THE 1996 CENTRAL SCOTLAND OUTBREAK OF *ESCHERICHIA COLI* O157:H7: INVESTIGATION OF CLINICAL PRESENTATIONS AND CONSEQUENCES

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Declaration

I declare that this thesis has been composed by myself. The research on the retrospective studies of hospitalised patients and the prospective studies of outbreak cases was entirely my own. I was assisted in the laboratory studies by Dr Caroline Blackwell and the Infection and Immunity Laboratory at Edinburgh University. I made a substantial contribution to the retrospective study of the community clinic. Dr Rachel Wood investigated the community clinic in greater detail and her research was presented to the University of Glasgow for a MPH in 1999. I undertook and completed this thesis in Edinburgh, during my Specialist Registrar Training in Infectious Diseases. The thesis has not been submitted for any other professional qualification.

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6th May 2002

Acknowledgments

During this research I have been privileged to receive the guidance and support of experts in the clinical and laboratory disciplines. I am especially grateful to Dr Andrew Todd whose dedication and enthusiasm was the catalyst to this project, and to Dr Caroline Blackwell who guided me through to the conclusion.

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Finally and in particular I would like to thank the patients and those who agreed to participate as control subjects. For many the outbreak was associated with personal loss. They gave their time voluntarily and without their assistance and goodwill none of this research would have been possible.

Abstract

In 1996 a large outbreak of *Escherichia coli* (*E. coli*) O157 occurred in central Scotland. The outbreak recorded the largest number of adults with HUS and consequently the largest number of deaths, ever attributed to *E. coli* O157. This unfortunate event provided opportunity to investigate severe acute disease and the chronic sequelae of infection. The retrospective studies aimed to assess host factors associated with the development of the haemolytic uraemic syndrome (HUS), to identify the earliest laboratory predictors of HUS, to develop a community monitoring protocol able to detect those with early HUS, to define the role of therapeutic plasma exchange (TPE) in the treatment of adults with HUS and to determine genetically mediated inflammatory responses associated with the severity of acute disease. The studies of chronic disease investigated renal function abnormalities in cases surviving HUS, and gastrointestinal complications and quality of life (QOL) in all cases. 512 cases were provisionally identified and 270 were confirmed by stool culture. 120 cases were admitted to hospital, 34 developed HUS and 17 died. Children and older adults were at greatest risk of HUS. However high white cell count (WCC) at presentation was as least as good a predictor of HUS as age. Acquired risk factors for HUS were low gastric acid and antibiotic therapy prior to symptom onset. 186 patients were assessed for blood group markers (ABO, Lewis and P). Blood group O and absent or weak expression of the P1 erythrocyte antigen were associated with HUS. Very high levels of TNFα, implicated in the pathogenesis of HUS, were produced by leucocytes of P-negative individuals. Adults in Lanarkshire who developed HUS were treated with TPE, which is unproven and

controversial in the context of *E. coli* O157. The mortality associated with HUS was 45%, which compares favorably with previously reported mortality of 88%. Therefore TPE appears to be a promising treatment. Thirty per cent of children develop chronic renal disease following VTEC induced HUS but there was no information on the renal outcome of adults. In Lanarkshire 12 of 22 adults with HUS survived the acute illness. To the third anniversary chronic renal disease was demonstrated in all; one progressed to ESRD, three developed CRF and eight had clear evidence of renal insufficiency. Comparison with a control group confirmed that these changes could not simply be attributed to age. Prospective investigation determined the prevalence of irritable bowel syndrome (IBS) after *E. coli* O157 infection and its impact QOL. On the second and third anniversaries of infection IBS was significantly higher in cases compared to matched controls. Almost forty per cent of cases developed new IBS within three years of infection. Cases with IBS had significantly lower SF-36 scores in all scales particularly those reflecting mental health. Therefore IBS is common after *E. coli* O157 with a detrimental effect on QOL. This thesis provides new insight into the pathogenesis and clinical consequences of disease due to *E. coli* O157, particularly in adults a previously undocumented group.

Abbreviation

ACMSF	Advisory Committee on the Microbiological Safety of Food
Ccr	Creatinine clearance
CDC	Centers for Disease Control and prevention
CHI	Community Health Index
CNS	Central nervous system
CRF	Chronic renal failure
CRP	C Reactive protein
DBP	Diastolic blood pressure
E. coli O157	*Escherichia coli* O157
EaggEC	Enteroaggressive *E. coli*
EHEC	Enterohaemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
EMU	Early morning urine
EPEC	Enteropathogenic *E. coli*
ESRF	End stage renal failure
ETEC	Enterotoxigenic *E. coli*
FFP	Fresh frozen plasma
Gb3	Globotriaosylceramide
GFR	Glomerular filtration rate
HUS	Haemolytic Uraemic Syndrome
IBS	Irritable bowel syndrome
IL	Interleukin
IMS	Immunomagnetic separation

ISD	Information and Statistics Division
LDH	Lactate dehydrogenase
LEE	Locus of enterocyte effacement
LPS	Lipopolysaccharide
PFGE	Pulsed field gel electrophoresis
PHLS	Public Health Laboratories Service
PT	Phage type
QOL	Quality of life
SBP	Systolic blood pressure
SCIEH	Scottish Center for Infection and Environmental Health
SMAC	Sorbitol MacConkey Agar
SNBTS	Scottish National Blood Transfusion Service
Stx	Shiga toxin
TMA	Thrombotic microangiopathy
TNFα	Tumour necrosis factor α
TPE	Therapeutic plasma exchange
TTP	Thrombotic thrombocytopenic purpura
VT	Verotoxin
VTEC	Verocytotoxin producing *E. coli*
WCC	White cell count

Contents

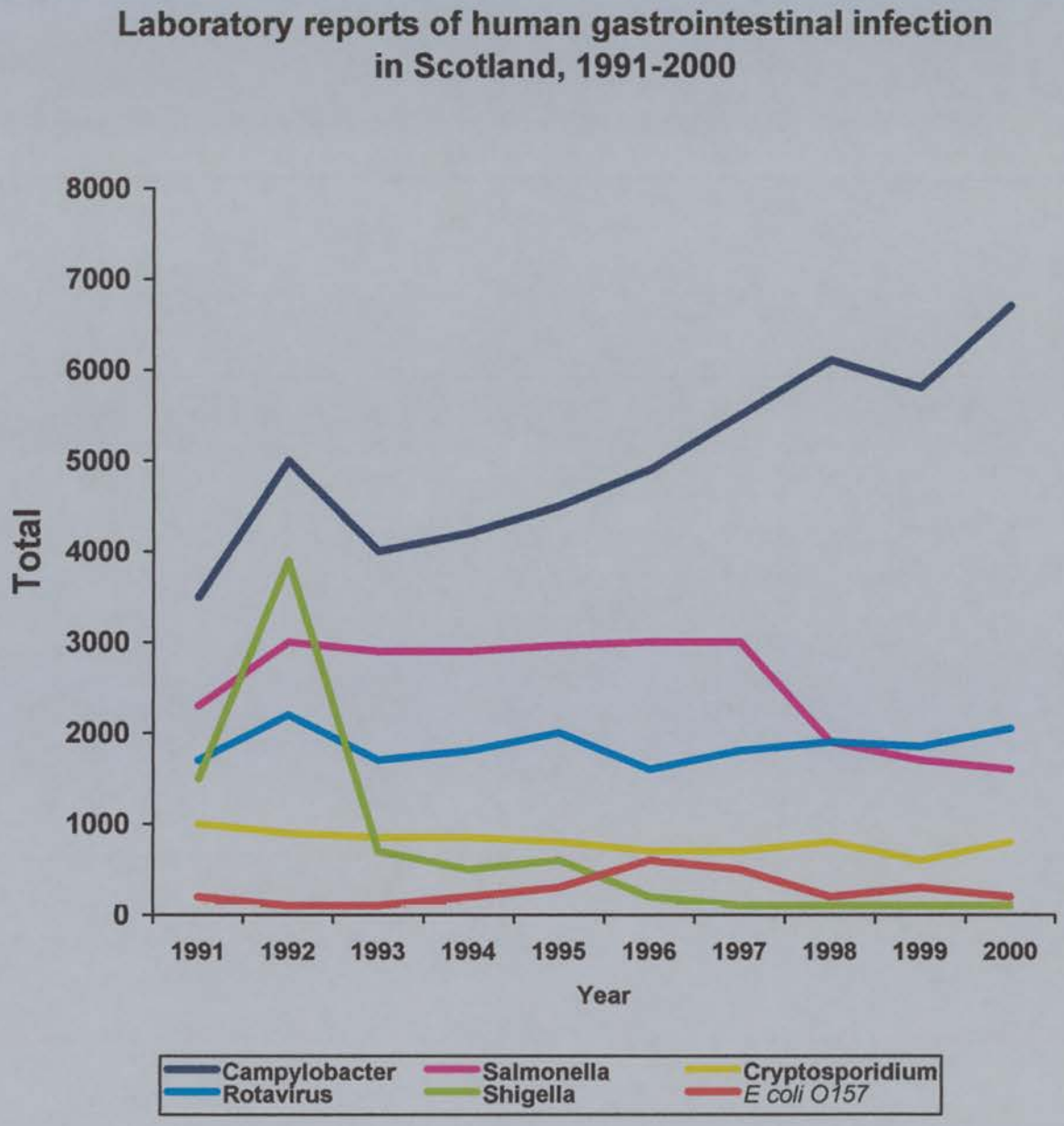
CHAPTER 1

General Introduction

1.1 Introduction

In terms of recognition and impact on public health policy verocytotoxin producing *Escherichia coli* (VTEC), and *Escherichia coli* O157 (*E. coli* O157) in particular, are still emerging pathogens, causing concern because of the severity of their complications. First recognised as a cause of haemorrhagic colitis in 1982 [Riley *et al.*, 1983], in subsequent years reported outbreaks continued to form a steeply inclined epidemic curve. The factors underlying the emergence of VTEC are not entirely clear. Increased incidence and recognition of infection are contributory, certainly *E. coli* O157 is not a new pathogen [Day *et al.*, 1983]. Recently the rise in infection has perhaps been tempered and this is a tribute to the success of improvements in surveillance, food handling, farm and slaughterhouse practices. Scotland still has one of the highest rates of *E. coli* O157 infection in the world, although comparatively *E. coli* O157 accounts for only a small proportion of all food poisoning notifications (Figure 1.1). The morbidity associated with *E. coli* O157 (31% of cases requiring hospital admission and an overall mortality rate of 3.7%) greatly exceeds that seen in infectious intestinal disease caused by the other pathogens (1.6% admissions and 0.1% mortality) [PHLS, 2000]. The severity of disease and well publicised incidents make *E. coli* O157 the subject of intense scientific research. Current interest is centred on the host susceptibility to severe disease, clinical management of acute infection, chronic sequelae and at a molecular level the mechanisms by which the verotoxins trigger the process that leads to the haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP).

Figure 1.1



[Scottish Centre for Infection and Environmental Health, (SCIEH) 2002]

1.2 Epidemiology of *E. coli* O157 in Human Disease

1.2.1 History of infection

In 1982, an investigation by the Centres for Disease Control and Prevention (CDC) of two outbreaks of severe bloody diarrhoea in the United States led to the identification of a strain of *E. coli*, one that expressed O- antigen 157 and H-antigen 7 that had not previously been recognised as a pathogen [Riley *et al.*, 1983]. The public health laboratories in the United States, the UK and Canada reviewed the serotypes of the *E. coli* isolates that had been sent to them over the previous decade. Since 1973, the CDC had serotyped over 3000 specimens of *E. coli*, only one of these, a 1975 isolate from a Californian woman with grossly bloody diarrhoea, was of serotype O157:H7. The PHLS in the UK reported that it had found this serotype in one of 15000 samples, Canada on the other hand found this serotype in six of 2000 isolates sent to them during the same years. Subsequent studies showed that sporadic cases of the organism were widely distributed [Remis and Ruggenti, 1995, Pai *et al.*, 1984] and analysis of multilocus enzyme electrophoresis data has shown that the evolution of *E. coli* O157:H7 probably began on the order of 5 million years ago [Whittam, 1995]. Unfortunately it cannot be determined when this organism acquired sufficient virulence to cause disease in humans, nor can one determine when it emerged in animal populations.

1.2.2 Reservoirs of infection

A bovine reservoir of *E. coli* O157 infection has been suspected since the first human outbreak was linked with ground beef consumption. Prior to 1982, this serotype had never been detected in veterinary samples. The organism has now been shown to be present in the gastrointestinal tract of cattle where it is non-pathogenic. Estimates of the prevalence of *E. coli* O157 in North American and European cattle range from less than 1% to almost 10% [Chapman *et al.*, 1993]. The organism has more recently been detected in small numbers of sheep [Chapman *et al.*, 1996] goats, horses, dogs, geese, pigs and gulls, suggesting that its animal reservoir is extending. *E. coli* O157 can survive for over 12 months in cattle faeces and over 20 weeks in soil samples [Maule, 1999].

1.2.3 Vehicles of transmission

Beef [Mead and Griffin, 1988, Wall *et al.*, 1996], particularly undercooked ground beef, and dairy products [Djuretic *et al.*, 1997] remain the most likely sources of infection. The infective dose of *E. coli* O157 is small and consequently cross contamination is increasingly recognised as an important source of infection. Cooked meats [SCIEH, 1997], radish shoots [Gutteriaz, 1997], potatoes, apple cider, mayonnaise, lettuce and drinking water have all been implicated as vehicles in outbreaks. Cross contamination by beef or contamination with bovine faecal material has often been suspected as the route of transmission. Outbreaks involving acidic foods, previously thought to be at low risk of transmission of pathogenic material, have underscored the unusual acid tolerance of this organism. Direct or indirect

contact with animals provides an alternative route by which infection is acquired [Trevena, 1996]. The median duration of shedding of the organism is 29 days [Shah *et al.*, 1996], it varies inversely with age and secondary attack rates of up to 22% are reported in young children [Belongia *et al.*, 1993]. High rates of person to person spread [Griffin and Tauxe, 1991] are important in causing outbreaks in schools, long term care institutions, families and day-care facilities.

1.2.4 Patterns of infection

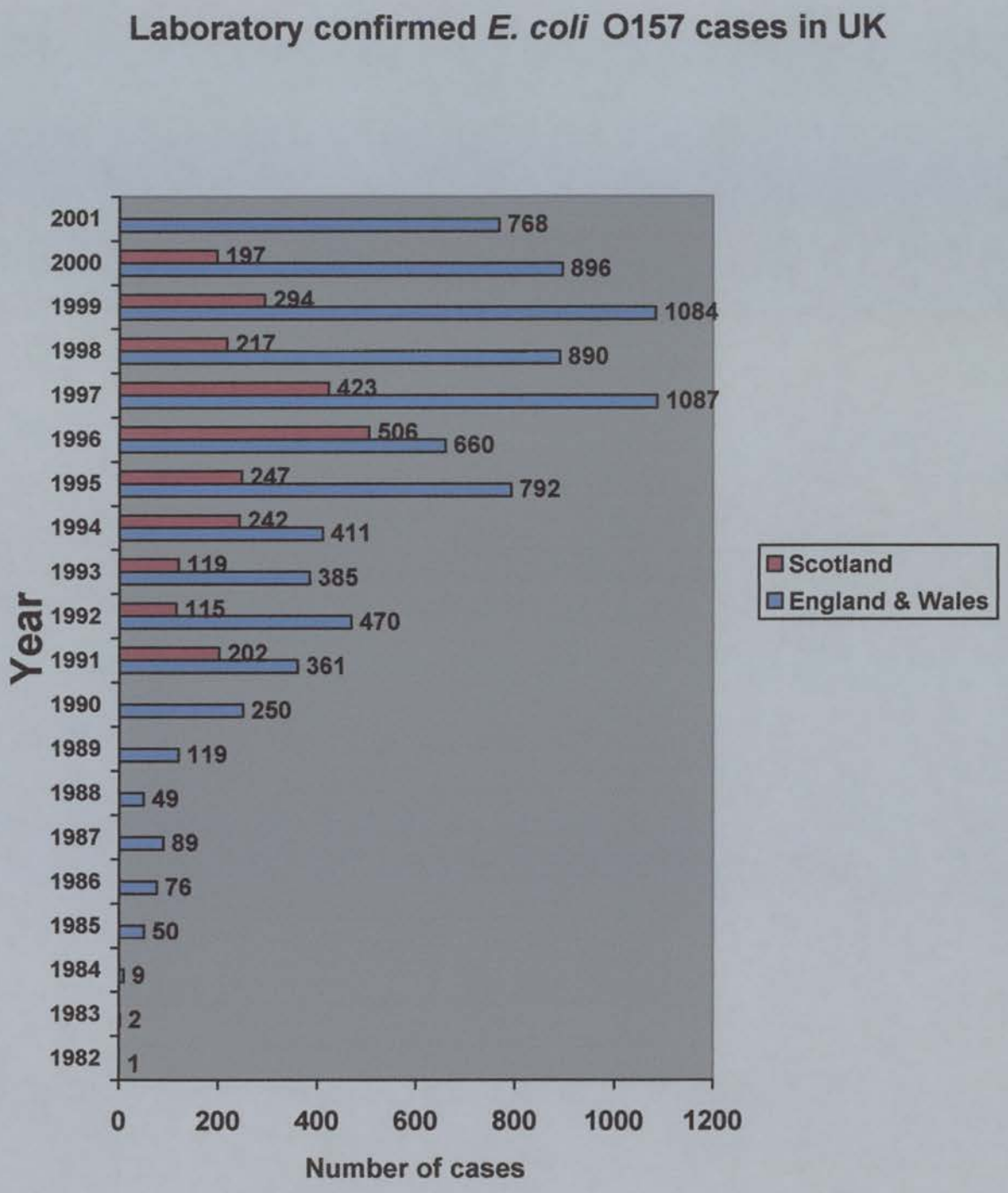
The livestock and beef industries changed dramatically during the past few decades and changes in slaughterhouse practices and both commercial and domestic food handling practices have all been implicated in the rise of infection. While no one change can be singled out as a major contributing factor to the emergence of *E. coli* O157, it is possible that these changes, have created a setting in which this organism has been able to spread more readily into and through animal and human populations. At the same time it is possible that the organism would have emerged as a major pathogen regardless of these changes, based on its ability to move into ecological niches in cattle and its virulence and low infectious dose in humans. Because the diagnosis and reporting of *E. coli* O157 has not been consistent over the last decade, it is conceivable that the observed increase in incidence of infection was artifactual. One direct gauge of the trend in infection is the incidence of HUS in children, which because of the severity of the illness is less prone to ascertainment bias. The number of HUS admissions in Scotland rose from 50 in 1980 to 250 in 1996, a rise paralleled by the number of *E. coli* O157 isolates [Douglas and Kurein, 1997], which would suggest that the actual incidence of infection was indeed rising.

There is a well-established seasonal trend in infection with a peak in summer each year [Milford *et al*,. 1991]. Most reported infection originally occurred in outbreaks and there was evidence that sporadic infection was significantly under recognised [Slutsker *et al.*, 1998]. The worst outbreaks were in Scotland (17 deaths) and in Japan (10000 cases and 5 deaths) and both occurred in 1996. The 1995 Government Advisory Committee on the Microbiological Safety of Food (ACMSF) and the 1997 Pennington report produced recommendations concerning methods of prevention of *E. coli* O157 transmission within foods and in the environment. These recommendations may have been instrumental in the decline in infection (Figure 1.2). Since 1997 there have been between 20 and 30 small outbreaks per year in the UK associated with unpasteurised milk, farms, cross contamination of cooked meats, beefburgers, and faecally contaminated raw vegetables and water. With implementation of the recommendation that laboratories test all diarrhoeal stools for *E. coli* O157 failure of recognition is less likely to remain a problem and 80% of reported infection is now sporadic (Figure 1.3).

1.2.5 Geographical distribution of infection

E. coli O157 has been isolated throughout the Western world particularly in Japan, the UK, United States, Canada, Argentina and Australia. Geographical distribution of infection in Scotland varies widely and incidence ranges from 0.9 to 15.1 per 100,000 [SCIEH 2002]. The incidence of infection in England and Wales ranges from 1.28-2.1 per 100,000 [Wilshaw, 2001]. In the United States the overall incidence is 1.65 per 100,000. Universally the incidence of infection is highest in children under 5 years.

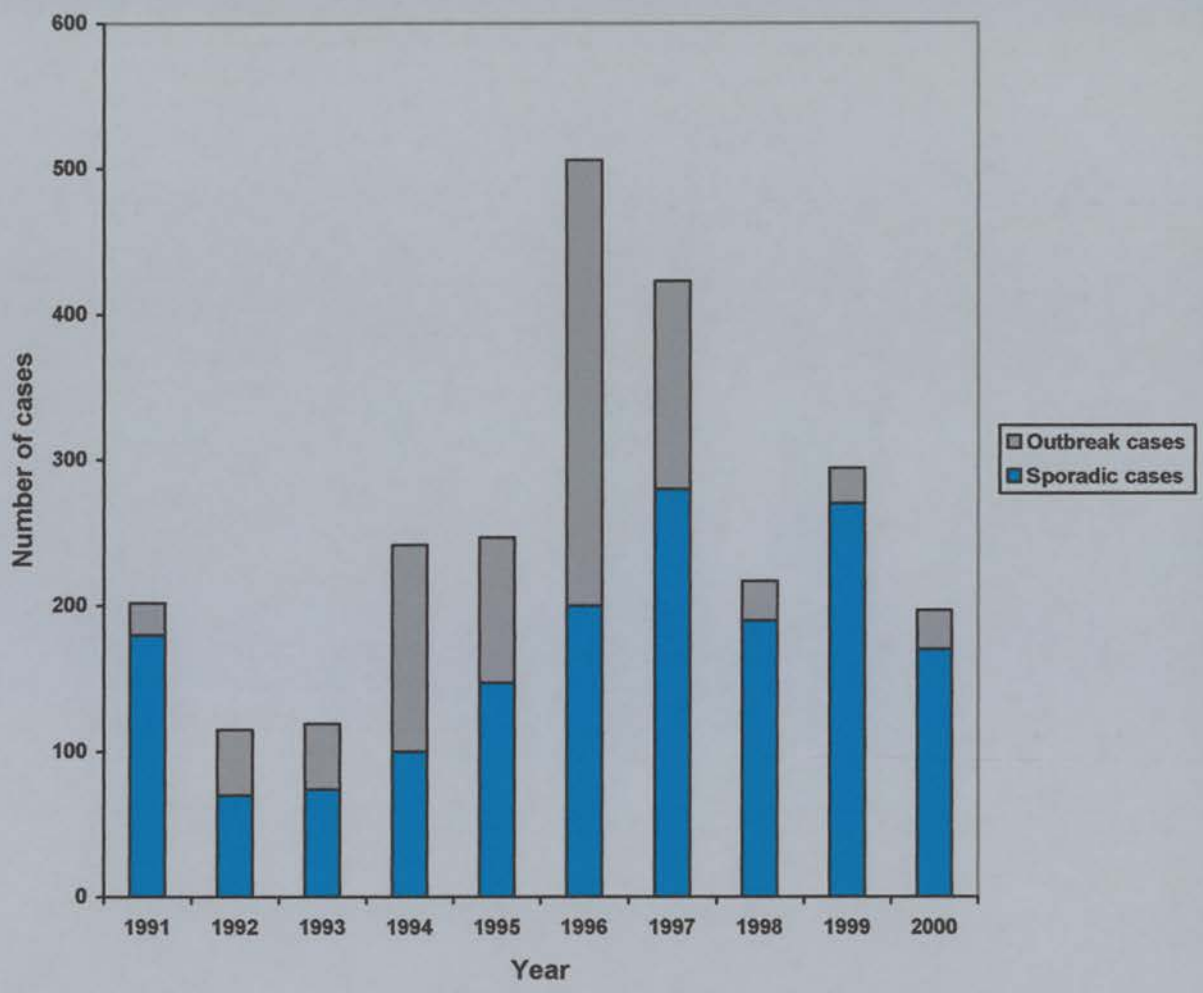
Figure 1.2



[Public Health Laboratory Service (PHLS) and SCIEH, 2002]

Figure 1.3

***E. coli* O157 human laboratory reports to SCIEH, outbreaks and sporadic cases**



[SCIEH, 2002]

1.3 The Organism

1.3.1 Classification of *E. coli*

E. coli, named after the German paediatrician Theodore Escherich who described it in 1885, is the most common aerobe among the normal human colonic flora and is also the most common human Gram-negative pathogen. *E. coli* belong to the family *Enterobacteriacea* and thereby share the following properties: they are gram negative rods, do not form spores, are motile by flagella or non motile, grow well on MacConkey agar, grow aerobically and anaerobically, ferment D-glucose, are catalase positive, reduce nitrate to nitrite, have 39 to 59 % guanine + cytosine content of DNA. Five distinct groups of *E. coli* cause gastrointestinal illness. VTEC, previously referred to as enterohaemorrhagic *E. coli* (EHEC), is the defined subset of verotoxin producing *E. coli. E. coli* O157:H7 and non O157 verotoxin producing *E. coli* belong to this group. Of the other four groups, enterotoxigenic *E. coli* (ETEC) produces cholera-like enterotoxins that elicit profuse watery diarrhoea, enteropathogenic *E. coli* (EPEC) causes infantile diarrhoea, enteroinvasive *E. coli* (EIEC) invades intestinal epithelium and produces dysentery and enteroaggressive *E. coli* (EaggEC) are more recently described, causing diarrhoea in children in the developed and developing world.

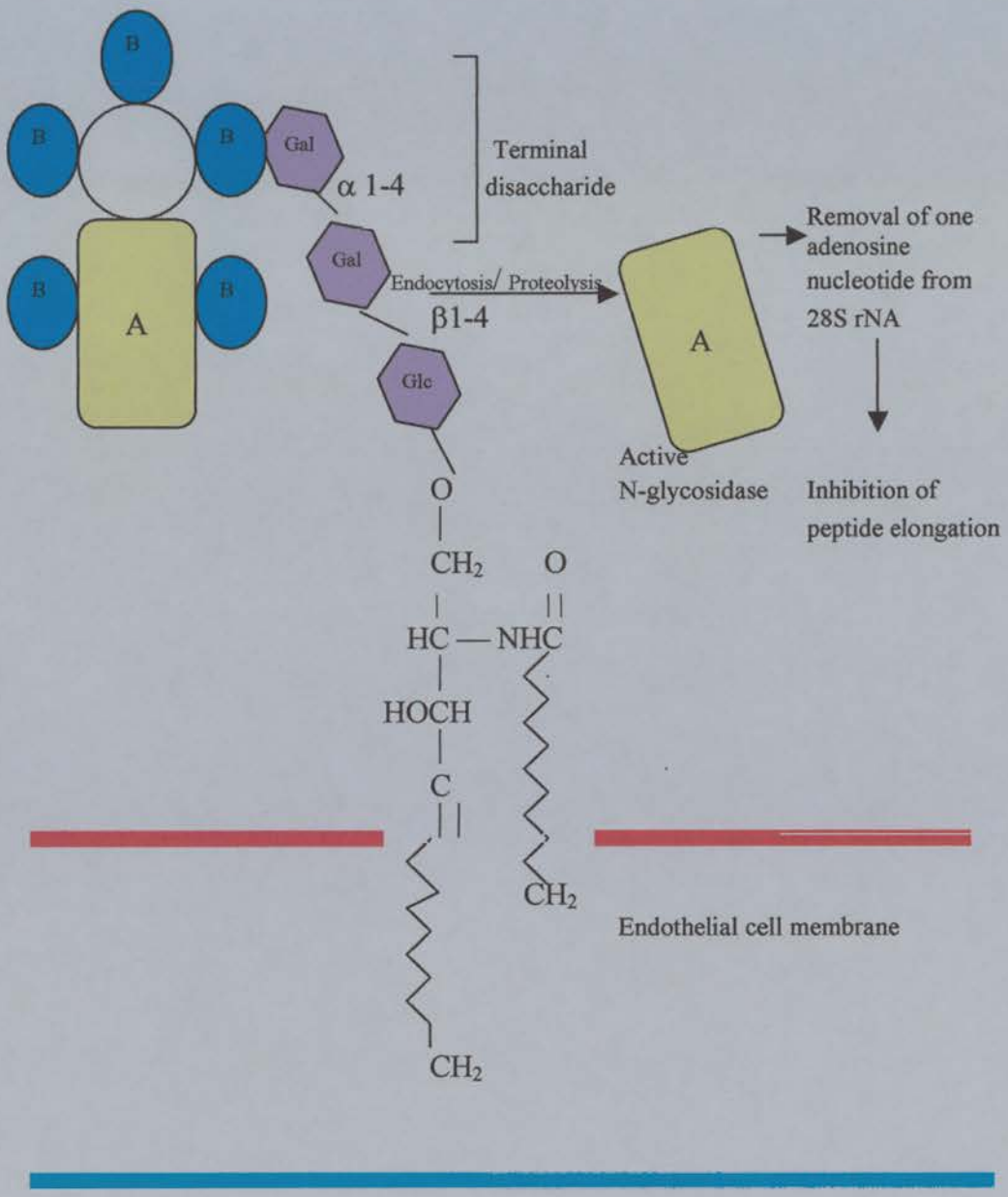
Three antigenic groups serotype *E. coli*: the lipopolysaccharide O antigens, the flagellar H antigens and the capsular K antigens. Approximately 170 O antigens and 60 H antigens are recognised. Following its ancestral divergence, *E. coli* O157:H7 lost its ability to ferment sorbitol and later its ability to produce β-D-glucuronidase.

It acquired verotoxin 2 (VT2), then verotoxin 1 (VT1) and possibly other virulence factors. The terminology used to describe the toxins produced by VTEC is confusing. Initially described as verotoxins because of their cytotoxic effect on vero cell lines, subsequently it was found that the toxin was immunologically, structurally and genetically almost identical to Shiga toxin from *Shigella dysenteriae* and therefore it was named Shiga toxin. Hence the terms verotoxin and Shiga toxin (Stx) are interchangeable, as are the terms vero-cytotoxin producing *E. coli* (VTEC) and shiga-toxin producing *E. coli* (STEC).

1.3.2 Pathogenesis

VTEC have three major pathogenicity mechanisms. They possess a plasmid-encoded adherence protein mediating early mucosal interaction. This is followed by attachment-effacement, identical to that of EPEC, mediated in part by intimin O157, an outer membrane protein [Louie *et al.*, 1993]. The intimin receptor has to be translocated from the bacterium to the mucosal cell. The genetic information for this process is housed on the genes for *eae/Tir* (enterocyte attachment and effacement translocated intimin receptor) part of the locus of enterocyte effacement (LEE) island. VT1 and VT2 are phage encoded enterotoxins and consist of an A and B subunit. The B subunit binds to the glycolipid receptor globotriaosylceramide (Gb_3) permitting endocytosis of the complex (Figure 1.4). Gb3 is particularly rich in renal glomeruli and the brain. After binding and internalisation of the toxin, the A subunit is dissociated and transferred from the Golgi apparatus to the endoplasmic reticulum, where it is cleaved into the A_1 and A_2 subunits.

Figure 1.4 : Binding of B subunits of verotoxins to renal microvascular endothelial cell via the disaccharide portion of Gb3 (Pk1) receptors



The toxic effect is mediated through the A_1 subunit which inhibits protein biosynthesis, by binding to the 60S ribosomal subunit, leading to apoptosis [Remuzzi and Ruggenti, 1995]. VT2 is 30 times more potent than VT1 [Nakao *et al.*, 1999]. Bacterial derived lipopolysaccharides [LPS] can act synergistically with verotoxins to initiate the inflammatory reaction in target organs by inducing the local production of inflammatory mediators like tumour necrosis factor α (TNF-α) or interleukins. In particular, enhanced TNF-α production might be pivotal in the pathogenesis of vascular injury by favouring neutrophil adhesion, with subsequent release of cytotoxic mediators, to the vascular wall [Lousi and Obrig, 1991].

Renal endothelial cell injury is probably the primary aetiological event in HUS. Swelling of the endothelial cells, widened subendothelial regions and hypertrophied mesangial cells combine to narrow the lumen of glomerular capillaries. Intraluminal platelet thrombi with some fibrin polymers occlude the narrowed glomerular capillaries and afferent arterioles [Habib, 1992]. In TTP arteriolar and capillary microthrombi have been demonstrated in the brain.

1.4 Diagnosis of Infection

1.4.1 Faecal sampling

E. coli O157 is the only serogroup of VTEC sought routinely by laboratories in the UK. Strains of *E. coli* O157:H7 are unable to ferment sorbitol rapidly and they are detected on sorbitol MacConkey agar (SMAC) on which they grow as colourless colonies. This has been used as a simple rapid means of screening stool specimens

for *E. coli* O157. Colourless colonies are confirmed as *E. coli* O157 by antiserum raised to the O-antigen. DNA probes have been prepared for both types of verotoxin gene, VT1 and VT2, and can be used to detect verotoxin in faeces. The probability of isolating *E. coli* O157 and detecting the verotoxins in stool from patients is inversely related to the interval between the onset of diarrhoea and microbiological culture [Tarr, 1995]. Improved detection methods are therefore being developed. Immunomagnetic separation (IMS) with anti-O157 beads is currently the most efficient and practical for clinical use [Karch *et al.*, 1996]. It allows the detection of small numbers of O157 strains (10^2 CFU/g of stool in the presence of 10^7 background flora); it is 10-100 times more sensitive than direct culture and less labour intensive than cytotoxic assay and PCR.

1.4.2 Serological diagnosis

In the past 10 years, the serological diagnosis of infection has been subject to much investigation. There is as yet no universally agreed technique. The current UK recommendation is that serum obtained one week after the onset of symptoms be examined for antibodies to the O157 lipopolysaccharide [LPS] antigen [PHLS, 2000]. Demonstration of rising antibody titres with paired specimens or detection of antibodies to O157 LPS by immunoblotting, which detects predominantly IgM, is a good indication of recent infection.

1.5 Clinical Features of Infection

1.5.1 Symptoms

The clinical features of the acute disease have been documented by the description of cases in outbreak situations. The incubation period for diarrhoeal illness is one to eight days. The spectrum of disease is wide ranging from the asymptomatic infection to death. Diarrhoea is the most common presenting symptom. Diarrhoea becomes bloody in 50% of cases. Abdominal pain also is common and often severe. Fever and vomiting are infrequent occurring in less than half of infected cases. The illness is usually self limiting within seven days. In the UK about a third of diagnosed cases are admitted to hospital [PHLS, 2000].

1.5.2 Complications

Gastrointestinal complications can be severe and ischaemic colitis, appendicitis, oesophageal stricture, intussusception and large bowel perforation are all described. Systemic complications were first recognised in 1985 when *E. coli* O157 was associated with the HUS [Karmali *et al.*, 1985] and later TTP [MMWR 1986]. HUS is characterised by the triad of acute renal failure, thrombocytopaenia and microangiopathic haemolytic anaemia. TTP was described when neurological symptoms and signs predominated over renal impairment. Thrombotic microangiopathy (TMA), the pathological basis of HUS, defines a lesion of small vessel wall thickening, intraluminal platelet thrombosis and partial or complete obstruction of the vessel. TMA presents as red cell haemolysis and thrombocytopenia and is a precursor to HUS, but it can occur and resolve in the

absence of overt clinical abnormality. The microvascular complications were shown to be mediated by the *E. coli* O157 toxins, VT1 and VT2 [Karmali and Tesh., 1991]. In recent years our understanding of the pathogenesis of HUS and TTP has evolved and both are now regarded as the same pathological and clinical entity, generally referred to as HUS [Todd, 2001].

Retrospective studies of outbreaks and sporadic cases have now shown that HUS occurs in 2-15% of cases of gastrointestinal infection with *E. coli* O157 [Griffin PM and Tauxe, 1991]. HUS develops almost exclusively in children and the elderly. The usual renal presentation of HUS is with oligoanuria, proteinuria and microscopic haematuria. The central nervous system (CNS) presentation of HUS ranges from restlessness to coma. Seizures are not uncommon. Imaging may show cerebral infarction or haemorrhage. Microvascular damage in the cerebral vessels, hyponatraemia, thrombocytopaenia and hypertension all contribute to the CNS presentation. Acute mortality in children has improved dramatically over the past two decades and is reported between 5 and 10% in most centres. Reported mortality in adults is much higher. The only significant adult cohort of *E. coli* O157 associated HUS, prior to the central Scotland outbreak, was from a Canadian nursing home where the reported mortality rate was 88% in 12 cases [Carter *et al.*, 1987].

1.5.3 Risk factors for systemic complications

The reason why HUS develops principally at the extremes of age is not clear but is likely to lie in the mechanisms by which the verotoxins mediate the microvascular thrombotic process. Verotoxin has been shown to bind to the glomeruli of infants

and not to the glomeruli of adults but there is poor correlation between the peak ages for HUS and the amount of Gb_3 present in the kidney. Age related differences in verotoxin absorption, glycolipid mediated verotoxin binding to Gb_3 receptors and rates of Gb_3 synthesis at the time of illness are suggested explanations for the discrepancy between verotoxin binding and Gb_3 receptor concentration [Arbus, 1997]. In *E. coli* O157 infection, HUS develops two to 14 days after the onset of diarrhoea. This provides a window of opportunity for laboratory monitoring and the identification of risk factors for the development of HUS. In children neutrophilia has been shown to be associated with progression to HUS. The value of other laboratory and clinical risk factors has been evaluated without overall conclusion.

1.5.4 Clinical management of the acute illness

Management of gastrointestinal infection is essentially that of symptom control and rehydration therapy with regular and careful monitoring for the development of HUS. Observations from outbreaks, controlled clinical trials and *in vitro* studies have failed to clarify the role of antibiotics in *E. coli* O157 infection, and consequently, the pattern of antibiotic use has changed little over the past 15 years. Particularly there is concern that antibiotics may increase the risk of developing HUS. Antimotility agents have been shown to be associated with HUS and should be avoided [Cimolai *et al.*, 1990, Akashi, 1994, Cohen, 1996].

A general consensus still credits the use of therapeutic plasma exchange (TPE) in adults with idiopathic HUS and all patients with TTP [Ruggenenti *et al.*, 1997], where it has clearly been shown to reduce mortality and chronic renal disease

[Conlon *et al.*, 1995]. In *E. coli* O157 associated HUS, the role of TPE in children is controversial and in adults there were no studies which reported its use. A prevailing criticism of plasma exchange is that it is started when the criteria for HUS are met and the microvascular process is already well established and perhaps beyond the stage for optimal intervention. The identification of markers which precede the development of HUS therefore has therapeutic implications.

1.6 The Host Susceptibility to Severe Disease

The wide spectrum of disease suggests that host factors are important in determining disease outcome. There is increasing evidence for genetic influences in proinflammatory responses such as TNFα and anti- inflammatory responses such as interleukin 10 (IL-10) to bacterial antigens, particularly endotoxin [Westendorp *et al.*, 1997]. Animal models indicate that endotoxin enhances the activity of verotoxins produced by *E. coli* O157. Inflammatory mediators markedly increase the binding of verotoxin to endothelial cells and may thereby play an important role in the pathogenesis of HUS. Individuals of blood group O have been shown to produce higher levels of TNFα, IL6 and nitric oxide in response to *Helicobacter pylori* [Alkout *et al.*, 2000]. It has been suggested that P1 red blood cell phenotype [Taylor *et al.*, 1990] and blood type B [Shimazu *et al.*, 2000] have a protective effect against VTEC associated HUS. Verotoxins have been shown to bind to the erythrocyte P1 antigen. P1 is a pentosyl ceramide that terminates, as does Gb3, in the galactose α1-4 galactose disaccharide recognised by the B subunits of verotoxin (figure 1.4). Whether or not P1 expression or genetically mediated inflammatory responses,

associated with blood group O, contribute to the severity of disease in patients with VTEC infection has not been resolved.

1.7 The Chronic Sequelae of Infection

1.7.1 Chronic renal sequelae

The legacy of *E. coli* O157 is now apparent. HUS is the commonest cause of chronic renal failure in children and accounts for 2.7% of children undergoing renal transplant in North America [Warady *et al.*, 1997]. Studies from children confirm that a spectrum of renal complications occur, at least in this age group. The results of the published reports on the late prognosis of HUS are difficult to compare for various reasons, but overall, 70% of children recover normal renal function and 30% have chronic renal abnormality of varying severity. There were no reports on the renal outcome of *E. coli* O157 associated HUS in adults.

1.7.2 Chronic gastrointestinal sequelae

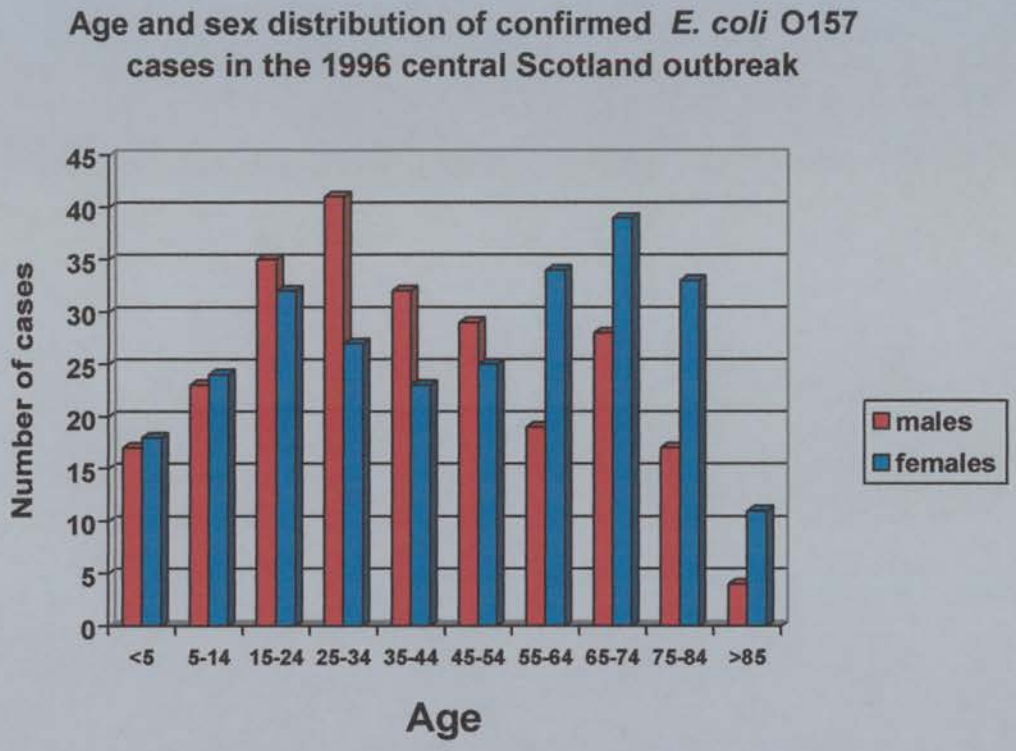
Symptoms secondary to bacterial gastroenteritis had traditionally been thought to be brief and self-limiting, but there is now growing evidence for the legacy of foodborne disease. Symptoms compatible with the irritable bowel syndrome (IBS) have been shown to occur after gastrointestinal infection with salmonella, campylobacter and shigella. IBS affects sleep, employment, sexual functioning, leisure, travel, diet and mood and consequently has a negative effect on quality of life [Hahn *et al.*, 1999]. The extent to which IBS occurs after infection with *E. coli* O157 was not known. Acute gastrointestinal symptoms and complications of *E. coli* O157 are often severe suggesting potential for chronic gastrointestinal problems and

late colonic stricture formation has been described [Griffin *et al.*, 1990]. Gall bladder disease requiring cholecystectomy, chronic pancreatitis and insulin dependent diabetes mellitus have all been described in a small number of children following *E. coli* O157 induced HUS.

1.8 The Central Scotland Outbreak

The central Scotland outbreak of *E. coli* O157 was identified on the 22nd November 1996 and declared over on the 20th January 1997. In this period 512 cases were identified and 279 confirmed by stool culture techniques. This remains the largest outbreak of *E. coli O157* infection in the UK to date [Cowden *et al.*, 2001]. The outbreak originated in a retail source in which cooked meats were cross contaminated. The Lanarkshire town of Wishaw was at the centre of the outbreak, but a second smaller outbreak was identified within the Forth Valley area. The first identified cohort were guests and helpers who attended a Christmas lunch for elderly parishioners on the 17th November 1996. Two other large cohorts within the outbreak were, guests who attended an 18th birthday party in Wishaw on the 23rd of November and residents and staff of a nursing home in Forth Valley who had eaten various sandwiches over the weekend 23-24th November 1996. A significant proportion of infected individuals were elderly, but ultimately the outbreak involved people of all ages (Figure 1.5). An outbreak monitoring clinic was set up to monitor cases in primary care.

Figure 1.5


Age and sex distribution of confirmed E. coli O157 cases in the 1996 central Scotland outbreak
Number of cases
45
40
35
30
25
20
15
10
5
0
<5
5-14
15-24
25-34
35-44
45-54
55-64
65-74
75-84
>85
Age
males
females

120 cases were admitted to three hospitals of these 34 developed HUS and 22 died (17 deaths were considered to be directly attributable to *E. coli* infection at the Fatal Accident Enquiry which followed the outbreak). The outbreak recorded the largest number of adult cases developing HUS and ultimately the greatest number of deaths attributed to *E. coli* O157.

This unfortunate circumstance provided a unique opportunity to investigate adults with uncomplicated gastrointestinal infection and HUS both during the acute illness and in the longer term.

1.9 Aims and Objectives

In this thesis, clinical and laboratory data from the outbreak and follow-up study of patients affected by *E. coli* O157 during the 1996 central Scotland outbreak will be presented and used to address the following questions.

1 What are the risk factors for HUS in adults infected with *E. coli* O157?

2 What are the earliest laboratory predictors of the development of HUS?

3 During a large outbreak can a protocol be developed for monitoring patients in the community that identifies those at risk of developing HUS?

4 Does therapeutic plasma exchange have a role in the treatment of adults who develop HUS in the context of *E. coli* O157 infection?

5 Are genetically mediated inflammatory responses of individuals associated with the severity of acute disease?

6 What is the incidence of chronic renal function abnormalities in adults after *E. coli* O157 induced microvascular disease?

7 Do patients develop IBS or other gastrointestinal complications after *E. coli* O157?

8 What is the impact of *E. coli* O157 infection on quality of life?

CHAPTER 2

General Subjects and Methods

2.1 Subjects

2.1.1 Retrospective study of outbreak

Subjects were cases identified during the 1996 Central Scotland outbreak of *E. coli* O157. The case definitions employed during the outbreak and the number of cases meeting each definition were as illustrated in Table 2.1. Subjects were eligible for inclusion if they met the confirmed, probable, or possible case definitions. For the community study confirmed, probable and possible cases were included. Confirmed cases were those in whom the outbreak strain was isolated from stool following standard culture on SMAC or after IMS. Provisional microbiological diagnosis was made in the laboratories at Law Hospital, Monklands Hospital and Falkirk Royal Infirmary. Isolates were confirmed and typed at the Scottish National *Escherichia coli* Reference Laboratory. The outbreak strain was *E. coli* O157 phage type (PT) 2, VT type 2 positive. All isolates were subsequently shown to be indistinguishable by pulsed field gel electrophoresis (PFGE). During retrospective analysis of the hospital records it became apparent that serology correlated poorly with stool culture confirmed infection, therefore serology was withdrawn from the case definition for the hospital studies. Probable was redefined as bloody diarrhoea or HUS and an association with implicated food source. Only confirmed and probable cases were included in the hospital studies. There were 512 cases of which 279 were confirmed. 380 cases were from the Lanarkshire area, 127 from Forth Valley and, four from Lothian and one from Greater Glasgow.

Table 2.1: Cases identified in the Central Scotland outbreak of *E. coli* O157

Symptoms	Stool specimen positive*	Stool specimen negative**	
		Serology positive***	Serology negative**
Asymptomatic or no history	**Confirmed (35)**	**Possible (53)**	**Not a case**
Non-bloody diarrhoea only	**Confirmed (77)**	**Possible (54)**	**Not a case**
Bloody diarrhoea and/or HUS	**Confirmed (167)**	**Probable (58)**	**Possible (68)**

*For the outbreak strain (*E. coli* O157:H7, phage type 2, verocytotoxin type 2 producing, DNA profile by pulse field gel electrophoresis characteristic of the outbreak strain) either by primary culture or immunomagnetic separation

**Or not done

***Or post mortem evidence of infection with the outbreak strain

2.1.2 Prospective study of outbreak cases

For logistical reasons the prospective study was limited to cases from the Lanarkshire area. Cases were those who had *E. coli* O157 infection during the central Scotland outbreak, who agreed on the first anniversary of infection to participate in a follow-up study. For adult cases, age and sex matched adult controls were recruited from the Community Health Index (CHI). CSC Scotland Healthcare Data Centre extracted from the CHI database 10 possible control subjects, of the same sex and year of birth, for each case. The permission of the individual's general practitioner was sought to approach the control subject and invite them to participate.

2.2 Methods

2.2.1 Retrospective study of outbreak

All the data relating to cases identified during the outbreak were validated and linked by the Information and Statistics Division (ISD) of the NHS in Scotland (Allan A, 1997). The data include patients' demographic features, microbiological, biochemical, and haematological results. We developed a comprehensive clinical database for all patients admitted to hospital. Data were collected from the hospital case notes in Monklands Hospital and Law Hospital in Lanarkshire, and Falkirk Royal Infirmary. Data were collected on premorbid illness, regular medications, symptoms, signs, management and complications of the acute illness.

2.2.2 Prospective study of outbreak cases

Cases and control subjects were reviewed annually by a research nurse, at community clinics in Lanarkshire Health centres, to the third anniversary of infection. Those unable to attend the clinic were visited at home. Annual review took place in November and December of each year and ended in 1999. Assessment of cases and controls ran simultaneously. All cases had a standard set of laboratory and clinical investigations carried out. These same tests were carried out on controls. Analysis of all serum and urine samples was carried out at Law Hospital Laboratories in Lanarkshire.

Adult cases and controls were asked to complete a quality of life questionnaire (SF-36) and an IBS questionnaire, both of which are self-completion questionnaires. A research nurse obtained history of new events and medications. All results were communicated to participants and their general practitioners.

2.2.3 The laboratory study

The laboratory study was carried out at the Infection and Immunity laboratory in the Department of Microbiology at Edinburgh University. Blood for the laboratory investigation was collected prospectively at the Lanarkshire clinics. For cases who had died, blood groups were determined from the hospital notes.

2.3 Definition of HUS

During the outbreak and throughout this study a consistent definition of HUS was used. Patients were required to have all three of the following: red cell fragmentation on blood film and lactate dehydrogenase >1.5 times the upper limit of normal; thrombocytopenia (platelet count $<150x10^9/l$); acute renal impairment (urea and creatinine above the reference range and rising) and/or new neurological signs.

2.4 Statistical Analysis

Statistical analysis was carried out using SPSS (version 7.5 and version 9). Epi Info was used for analysis of the laboratory study. CHI squared test and students t-test (2 tailed) were applied to examine statistical differences between groups. $P < 0.05$ was considered statistically significant and Odds Ratio (OR) >1 taken to equal association. Assistance in multivariate statistical analysis was given by the Scottish Centre for Infection and Environmental Health (SCIEH).

2.5 Ethical Approval

Subjects were provided with Information Sheets and they signed consent forms for the prospective study. Ethical approval was granted by Lanarkshire Health Board Research Ethics Committee.

CHAPTER 3

Monitoring Patients in the Community with Suspected *E. coli* O157 Infection During the Central Scotland Outbreak

3.1 Introduction

Early in the course of the central Scotland outbreak the source of infection was traced to a butcher's shop in Wishaw, a town with a population of approximately 50,000. The majority of cases lived in Wishaw and the surrounding area and understandably there was a great deal of public anxiety about the outbreak within the town. To ease resulting overwhelming pressure placed on local primary and secondary care services a special community outbreak clinic was quickly established in the local health centre. The clinic aimed to support the public health investigation into the outbreak, facilitate the provision of consistent public health advice to minimise secondary spread of the infection, and co-ordinate the care of cases.

The outbreak clinic was principally used to monitor patients from the local community with clinically suspected *E. coli* O157 infection. A patient with onset of diarrhoeal illness since the start of the outbreak was defined as having suspected infection and these patients could be referred to the clinic by their General Practitioners. In addition the clinic was also used by patients required to submit samples for work exclusion purposes, and to follow up after discharge a number of known cases who were admitted to hospital early in the course of the outbreak.

A protocol to guide the management of patients with suspected *E. coli* O157 infection was drawn up at the start of the outbreak and was used consistently at the clinic. The protocol specifically aimed to identify patients' case definition status,

ensure early diagnosis of HUS, and facilitate prompt and appropriate referral of patients requiring secondary care.

Stool microbiology and paired serology were performed to establish patients' case definition status. Stool culture on SMAC was performed at the local laboratory, however confirmatory tests were carried out at the Scottish *E. coli* O157 reference laboratory in Aberdeen. Due to the inevitable time delays involved in obtaining final results from the reference laboratory, at the time patients were attending the clinic their final case definition was often still unknown.

To monitor for HUS all patients with suspected infection had their haemoglobin, white cell count (WCC), platelet count, blood film, lactate dehydrogenase level (LDH), and serum urea and creatinine checked every two days for 14 days. Standard criteria were developed to guide referral to secondary care. Referral was recommended for patients with severe clinical manifestations of infection (such as dehydration requiring parenteral fluid management) and patients whose laboratory findings indicated the imminent development of HUS. A full account of the running of the clinic including details of the management protocol used are given in the Outbreak Report produced by Lanarkshire Health Board [LHB, 1999].

Previous studies have identified risk factors for HUS in patients with known *E. coli* O157 infection. Young children and the elderly have consistently been found to be more likely to develop HUS than young adults [Su and Brandt, 1995,Griffin and Tauxe, 1991,Carter *et al.*, 1987]. Studies involving children have suggested that a

raised WCC early in the course of illness is also associated with an increased risk of HUS [Pavia *et al.*, 1990, Bell *et al.*, 1997, Akashi *et al.*, 1994]. Reports regarding gender as a risk for HUS have been less consistent [Su and Brandt, 1995, Griffin and Tauxe, 1991, Bell *et al.*, 1997].

The database developed by ISD in Scotland provided the data for the study [Allan *et al.*, 1997]. Data examined included patients' demographic features, and all microbiological, biochemical, and haematological results. The data provide a unique opportunity to examine the features associated with the subsequent development of HUS in patients from a wide age range with suspected *E. coli* O157 infection.

In accordance with what is known about features associated with the development of HUS in patients with known *E. coli* O157 infection we have focused our analysis on the ability of age and patients' WCC to predict the development of HUS in suspected infection. The study of the community clinic did not examine other clinical features, such as clinical features and antibiotic use, in detail. This is due to a lack of information on such issues being recorded at the community clinic.

3.2 Subjects and Methods

Patients with suspected *E. coli* O157 infection (but not in established HUS) who were referred from primary care to the Wishaw clinic for monitoring were included in the study.

The case definitions employed during the outbreak and the number of cases meeting each definition in the community clinic study were as illustrated in Table 3.1. In this study a case was defined as any patient meeting the confirmed, probable, or possible case definitions.

For the purposes of this study the outbreak definition of HUS was used. Patients were required to have all three of the following:

- Red cell fragmentation on blood film, and lactate dehydrogenase >1.5 times the upper limit of normal.
- Thromocytopenia (platelet count $<150x10^9/l$).
- Acute renal impairment and/or new neurological signs.

The day on which all three criteria were met are defined as the day of onset of HUS.

The socio-economic status of patients was assessed by converting postcodes of residence into deprivation categories using the Carstairs and Morris index [Carstairs and Morris, 1991]. Deprivation categories are ranked 1 to 7, with 7 representing the most deprivation.

We first assessed the association in patients with suspected infection between age, and gender and subsequent development of HUS. We next mapped the clinical course of the patients who developed HUS in detail to identify which laboratory parameters became abnormal first. We then specifically assessed the ability of the WCC to predict the development of HUS in patients with suspected *E. coli* O157 infection.

Table 3.1: Case definition of the 245 cases monitored at the community clinic

Symptoms	Stool specimen positive*	Stool specimen negative**	
		Serology positive***	Serology negative**
Asymptomatic or no history	Confirmed (19)	Possible (23)	Not a case
Non-bloody diarrhoea only	Confirmed (51)	Possible (15)	Not a case
Bloody diarrhoea and/or HUS	Confirmed (72)	Probable (28)	Possible (37)

*For the outbreak strain (*E. coli* O157:H7, phage type 2, verocytotoxin type 2 producing, DNA profile by pulse field gel electrophoresis characteristic of the outbreak strain) either by primary culture or immunomagnetic separation
**Or not done
***Or post mortem evidence of infection with the outbreak strain

The sensitivity, specificity, and predictive values of a patient being found to have one or more abnormally high WCC results prior to the onset of HUS were calculated. An abnormally high result was defined as one above the age appropriate reference range specified by the local laboratory (reference ranges for all laboratory parameters are available on request). The exact method for calculating a confidence interval for a single sample proportion was used to calculate 95% confidence intervals for all results [Armitage and Berry, 1987]. The analysis was repeated using neutrophil count rather than total WCC. Finally we assessed the ability of age and WCC considered together to identify patients at highest risk of developing HUS.

3.3 Results

3.3.1 Demographic data

In total 1,198 people presented to the Wishaw clinic. Of these 229 did not meet the clinic's criteria for assessment, for example they had self referred, and hence were given advice only. A further 36 people had no blood samples taken at the clinic, this group includes people required to submit microbiological samples only for possible work exclusion purposes. 933 people therefore underwent blood test monitoring according to the clinic protocol. 39 of the 933 patients were referred from hospital for post discharge follow up, for seven patients it was unknown whether they were referred from primary or secondary care. The remainder were known to be referred from primary care. One person referred from primary care was in established HUS at the time of referral to the clinic so no information could be obtained on the

features predating the development of their HUS. 886 patients were therefore eligible for inclusion in the study (figure 3.1).

We consider this group to be representative of patients presenting to primary care with suspected *E. coli* O157 infection during a community based outbreak. Of the 886 patients monitored at the clinic, 245 were found to be cases. 170 cases (69%) had confirmed or probable infection (table 3.1). 27 of the cases were admitted to hospital, nine developed HUS, and two died.

The age and gender distribution of the patients monitored at the clinic is shown in figure 3.2. A large proportion of cases monitored at the clinic were aged between 15 and 64 years and younger and older cases were under represented (figure 1.5). 89% of the patients monitored at the clinic were resident in areas assigned deprivation category five or six. This reflects the fact that Wishaw and its surroundings is a relatively deprived area of Scotland.

3.3.2 Predictors of HUS in all patients presenting

Men (3/346) and women (6/540) monitored at the clinic were equally likely to develop HUS (Fisher's exact test p=1.0). Children <15 years and adults >64 years (7/298) however were significantly more likely than adults aged 15-64 years (2/588) to develop HUS (Fisher's exact test p=0.008).

Figure 3.1: Community clinic patients eligible for inclusion in the study

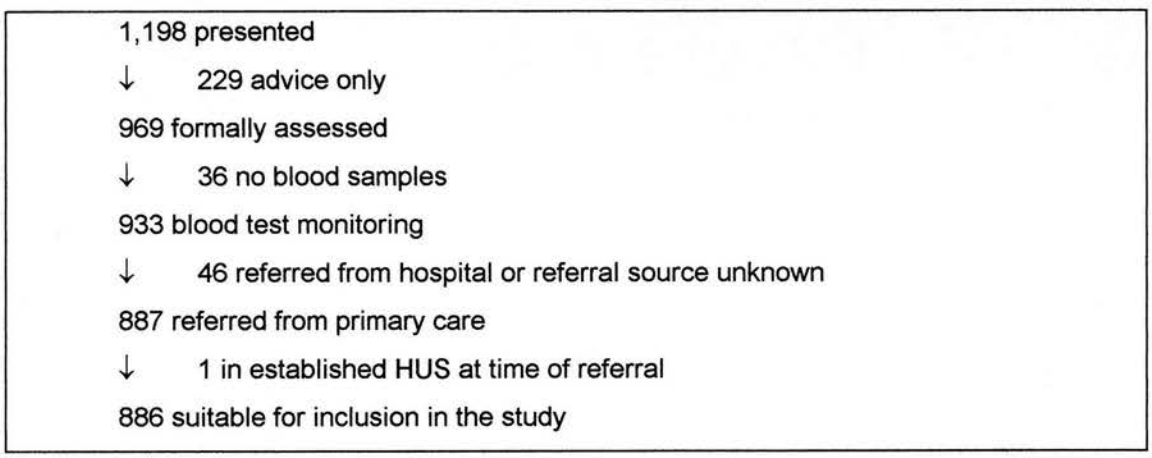
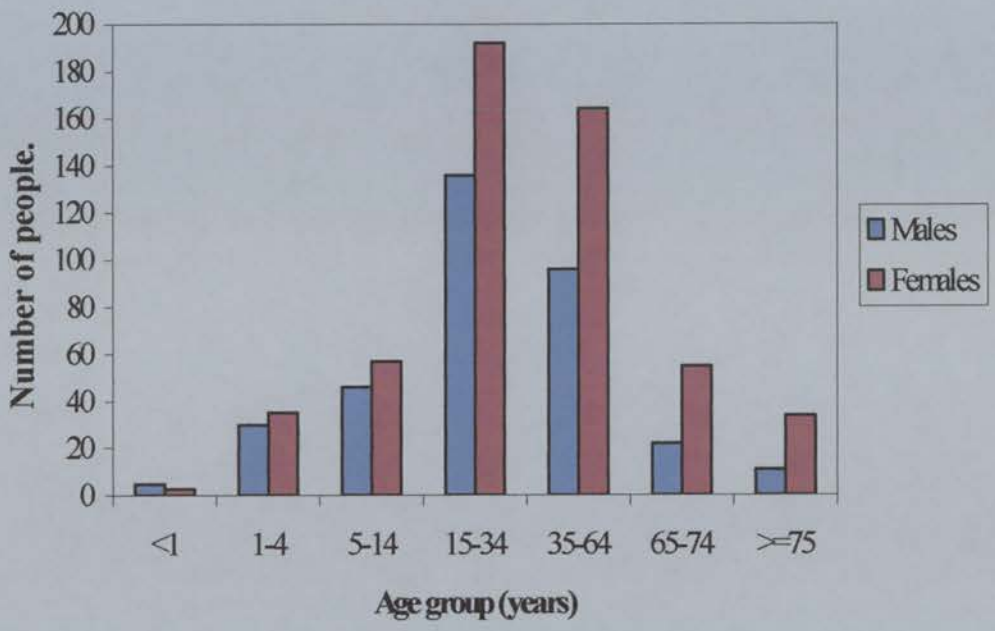


Figure 3.2: The age and gender distribution of patients monitored at the community clinic.



In terms of assessing the ability of laboratory parameters to predict HUS, we initially examined in detail the clinical course of the nine patients that developed complications (table 3.2). The median interval between onset of symptoms and onset of HUS was nine days (range 5 to 15). In general an elevated WCC preceded the development of HUS and also preceded changes in urea, creatinine, LDH, haemoglobin, and platelet levels, and the appearance of fragmented red cells (table 3.3).

Eight of the nine patients with HUS had a high WCC at some point during their illness. In all eight the WCC became abnormal before the onset of HUS, a median of 1.5 days after the onset of symptoms, and five days (range 1 to 8) before the onset of HUS. In seven of the eight patients, the exception being the patient who did not develop a raised WCC until 14 days after the onset of symptoms, the WCC was abnormal on the first blood sample obtained when the patients presented.

Having confirmed that WCC was the first laboratory parameter to become abnormal in patients developing HUS, we assessed the ability of a high WCC result to predict subsequent HUS in the whole group of patients monitored at the community clinic.

The presence of one or more high WCC results predicted the subsequent development of HUS with a sensitivity of 89%, specificity of 87%, positive predictive value of 7%, and negative predictive value of over 99% (table 3.4).

Table 3.2: Demograpic and laboratory details of the nine cases that developed HUS

Case Number	Age	Sex	Blood in stool	Case definition	Clinic	HUS	WCC	Haemoglobin	Fragmented red cells	LDH	Platelets	Urea	Creatinine
1	70	F	Yes	Confirmed	0	5	0	5	3	4	5	2	5
2	70	M	Yes	Confirmed	1	5	1	5	5	1	5	1	N
3	63	F	Yes	Confirmed	3	6	1	1	3	3	6	3	N
4	10	M	No	Confirmed	2	7	2	N	7	4	7	7	2
5	6	F	Yes	Confirmed	1	9	1	N	7	1	9	7	1
6	80	F	No	Confirmed	4	11	4	11	8	8	11	8	N
7	78	F	Yes	Probable	5	11	5	8	8	5	10	5	5
8	61	F	Yes	Confirmed	6	12	N	12	12	10	6	10	10
9	2	M	No	Confirmed	2	15	14	6	15	10	15	15	2

Clinic – day of first attendance at clinic
HUS – day of onset of HUS
All blood results – day of first recorded abnormal result (high or low as appropriate)
Note all results are based on the day of onset of symptoms being day 0
N indicates that no abnormal result for that blood parameter was recorded for that patient at any point during their illness

Table 3.3: Median interval between onset of symptoms and first abnormal result in cases with HUS

	Number of patients with an abnormal result recorded at some point during the course of their illness	Median interval between onset of symptoms and first recorded abnormal result (days)	Range (days)
WCC	8 / 9	1.5	0-14
Haemoglobin	8 / 9	7	1-12*
Fragmented red cells	9 / 9	7	3-15
LDH	9 / 9	4	1-10
Platelets	9 / 9	7	5-15
Urea	9 / 9	7	1-15
Creatinine	6 / 9	2	1-10

Day 0 is day of onset of symptoms
*Note that one of the 8 patients who developed anaemia only did so after the onset of their HUS

3.3.3 Predictors of HUS in cases

It is possible that diagnostic tests for *E. coli* O157 infection, more rapid than current stool culture techniques, may be developed in the future allowing primary care practitioners to readily distinguish patients with definite infection from those with other causes of gastrointestinal symptoms. For this reason we also assessed the ability of a high WCC to predict the subsequent development of HUS in the cases monitored at the clinic. The results were not significantly different to those for the whole group of patients with suspected infection (table 3.4).

As it is specifically neutrophils that are implicated in the pathophysiology of complicated *E. coli* O157 infection, in addition we assessed the ability of the neutrophil count to predict the development of HUS. We found that the neutrophil count was not significantly better than the total WCC in predicting HUS.

3.3.4 Predictive value of age and WCC combined

Finally, as we had identified age group and WCC as the features most strongly associated with the subsequent development of HUS in patients with suspected *E. coli* O157 infection, we assessed the predictive value of age group and WCC combined. The positive predictive value of age (<15 or >64) alone was 7/298 (2.3%, 95% CI 0.9-4.8%); that of WCC alone was 8/121 (6.6%, 2.9-12.6%); and that of age and WCC combined was 7/50 (14%, 5.8-26.7%).

Table 3.4: The validity of a high white count result in predicting subsequent development of HUS in all patients (cases) monitored at the community clinic

High white count	HUS	No HUS	Total
Present	8 (8)	113 (49)	121 (57)
Absent	1 (1)	764 (187)	765 (188)
Total	9 (9)	877 (236)	886 (245)

Summary of results for all patients
Sensitivity 88.9% (51.8 – 99.7%)
Specificity 87.1% (84.9 – 89.3%)
Positive predictive value 6.6% (2.9 – 12.6%)
Negative predicative value 99.9% (99.3 – 100%)

Summary of results for cases
Sensitivity 88.9% (51.8 – 99.7%)
Specificity 79.2% (74.1 – 84.4)
Positive predictive value 14.0% (6.3 – 25.8%)
Negative predicative value 99.5% (97.1 – 100%)

3.4 Discussion

This study is unique in assessing features associated with the development of HUS in a large number of patients, from a wide age range, with suspected *E. coli* O157 infection monitored in a community setting during a large scale outbreak.

Children and the elderly with suspected infection are at higher risk of developing HUS than young adults. This agrees with previous work involving patients with known *E. coli* O157 infection [Su and Brandt, 1995, Griffin and Tauxe, 1991, Carter *et al.*, 1987].

Significantly, this study also demonstrates the importance of a raised WCC as a predictor of HUS, in patients of all ages with suspected as well as known *E. coli* O157 infection. The study has shown that raised WCC is at least as good and possibly an even better predictor of HUS than age and that patients with normal WCC are at very low risk of HUS. Additionally it appears that WCC becomes abnormal before the laboratory changes which characterise HUS.

During the central Scotland outbreak the Wishaw clinic was invaluable in alleviating pressure on local services, and ensuring that patients received consistent information and were monitored and referred on to secondary care in a consistent way. In addition the clinic facilitated comprehensive data collection allowing subsequent

evaluation to be undertaken. We would recommend the establishment of a similar clinic during any large community based *E. coli* O157 outbreak.

The protocol used at the clinic however required very comprehensive monitoring of all patients with suspected infection, including patients that we could now identify as being at very low risk of developing HUS. In the future a streamlined monitoring protocol for patients in the community with suspected *E. coli* O157 infection, targeting those at extremes of age who present with high WCC, should be adopted.

CHAPTER 4

Risk factors for HUS and Death in all Cases Admitted to Hospital During the Central Scotland Outbreak of *E. coli* O157

4.1 Introduction

In the community identification of risk factors for HUS in patients presenting with suspected *E. coli* O157 infection allows monitoring to be targeted at those with greatest risk. In the hospital setting infection is largely already confirmed and the emphasis is on the earliest identification of factors which predict progression from gastrointestinal infection to HUS. This is especially true with the promise of new agents that may block verotoxin absorption to prevent HUS [Armstrong *et al*, 1995, Takeda *et al.*, 1999], and the theoretical value of early TPE as possible treatment. Extremes of age and raised WCC are the most consistently reported risk factors for HUS [Drummond, 1985, Griffin *et al.*, 1988, Carter *et al.*, 1987, Pavia *et al.*, 1990, Walters *et al.*, 1989, Milford *et al.*, 1991, Kawamura *et al.*, 1999]. Both were confirmed to be risk factors in the community study. There is disagreement regarding antibiotic therapy anti-motility agents, fever, bloody stools, vomiting, and gender as other potential risk factors in the progression to HUS [Carter *et al.*, 1987, Pavia *et al.*, 1990, Butler *et al.*, 1987, Cimolai *et al.*, 1990, Bell *et al.*, 1997, Proulx *et al.*, 1992, Takeda *et al.*, 1998, Shiomi *et al.*, 1999, Kimmit *et al.*, 1999, Ikeda *et al.*, 1999, Wong *et al.*, 2000]. To date, most reports relate to the paediatric population and features of adult disease are essentially unknown. Study of patients admitted to hospital during the central Scotland outbreak allowed detailed investigation of HUS in a predominantly adult group.

4.2 Subjects and Methods

4.2.1 Data collection

Data were collected, from the case notes of all cases admitted to hospital, on premorbid illness, regular medications, symptoms, signs, management and complications. Clinical data were linked to demographic and laboratory data retained on the ISD database.

4.2.2 Inclusion criteria

All "confirmed" and "probable" cases of *E. coli* O157 infection admitted to hospital during the central Scotland *E. coli* O157 outbreak were included. "Confirmed" cases were those in whom the outbreak strain was isolated from stool by standard culture with or without IMS [Karch *et al.*, 1996]. "Probable" cases were those with bloody diarrhoea or HUS and an association with implicated food sources, but no *E. coli* O157 or other organism isolated. Retrospective analysis of serology performed during the outbreak found it to correlate poorly with stool culture and clinical diagnosis of infection, therefore to ensure that only true cases were included within the hospital study, serology was withdrawn from the case definition and "possible" cases were excluded.

4.2.3 Outcome measures

The definition of HUS was as in the outbreak and the community study. One patient, who met all other criteria, was included as having developed HUS despite a minimum platelet count of 228 (on death).

4.2.4 Analysis

Demographic features, clinical symptoms and signs, antibiotic therapy, pre-morbid illness, regular medication, pre-admission treatment and laboratory variables were assessed in relation to outcome measures (HUS and death) using univariate and multivariate logistic regression analysis. The dichotomization of continuous variables was achieved based on either examination of their distribution, which yielded a natural cut off, or from examples of categories used in previous studies. Due to limited sample size, only variables found to be significantly associated with outcome measures in the univariate analysis were included in the multiple regression model.

Symptoms and signs were taken as those recorded prior to, or within 24 hours of admission. Fever was defined as axillary temperature > 37.5°C recorded within 24 hours of admission. *Antibiotic therapy* was included only if it was initiated within four days of symptom onset. During the outbreak all cases admitted to one hospital site were asked specifically about *coincidental antibiotic use* (prescribed for another infection within four weeks prior to developing *E. coli* O157 related symptoms) and the use of *antimotility drugs*. This information was not recorded at other hospitals and analysis is confined to patients who were admitted to the hospital where the data were prospectively collected. *Chronic pre-morbid illness* and *regular medication* were only assessed in adults (> 15 years of age), as no children had pre-morbid illness or were taking regular medication.

This outbreak was unique in that laboratory data were monitored in all possible cases from presentation until day 14 of illness at least, in hospital or in the outbreak monitoring clinic. WCC, neutrophil count, haemoglobin, albumin, LDH, urea and creatinine were assessed as predictors of HUS in hospitalised cases using results at first blood sample. Due to the potentially rapid development of HUS (3 days in one case) only cases with a first blood sample within 48 hours of the onset of symptoms were included. This restriction limited the sample size, therefore multivariate analysis was not performed on this data. Further investigation of laboratory data (for the first 14 days of illness), in all cases, was performed by examining the mean value of variables per day after onset of symptoms, in the group who developed HUS and those who did not. The t-test for equality of means was applied to detect the first day in which there was a significant difference in each laboratory variable, between the two groups.

4.3 Results

345 confirmed and probable cases of *E. coli* O157 were identified in the Lanarkshire and Falkirk areas. 279 (81%) cases were confirmed by stool culture techniques. 120 (35%) cases were admitted to three primary hospitals; two hospitals in Lanarkshire and one in Falkirk. It is this group that was investigated in the regression analyses. Age range of admitted cases was 18 months to 94 years, with a median age of 63 years. Admissions occurred between 20th November 1996 and 16th December 1996.

4.3.1 Complications

HUS: 34 cases developed HUS, 26 adults and eight children. HUS developed a median of 7 days (range 3-15 days) after the onset of gastrointestinal symptoms. 12 of 26 adults and 1 of eight children had neurological complications of HUS, the most frequent of which was cerebrovascular accident.

Death: 16 (13%) hospital admissions died. All were aged over 65 years. 11 had complete HUS, three had an incomplete form of HUS and two had no evidence of microvascular complications. Seven of the 11 patients with HUS who died had neurological complications. The mortality rate in adults with HUS was 42% (11 of 26).

4.3.2 Treatment

The mainstay of treatment was supportive with correction of fluid and electrolyte imbalance. Antibiotic therapy for the treatment of gastroenteritis was prescribed according to UK guidelines; therefore ciprofloxacin was given to cases aged over 60 years with pre-existing medical conditions or taking immunosuppressive therapy, acid lowering drugs or ACE inhibitors [Farthing *et al.*, 1996]. 20 of 120 cases received ciprofloxacin, no other antibiotic was given as primary treatment. TPE was carried out in 16 adults in one Health Board area and one adult outwith this area. Five adults so treated had progressive renal impairment requiring haemodialysis. Seven children received dialysis.

4.3.3 Predictors of HUS

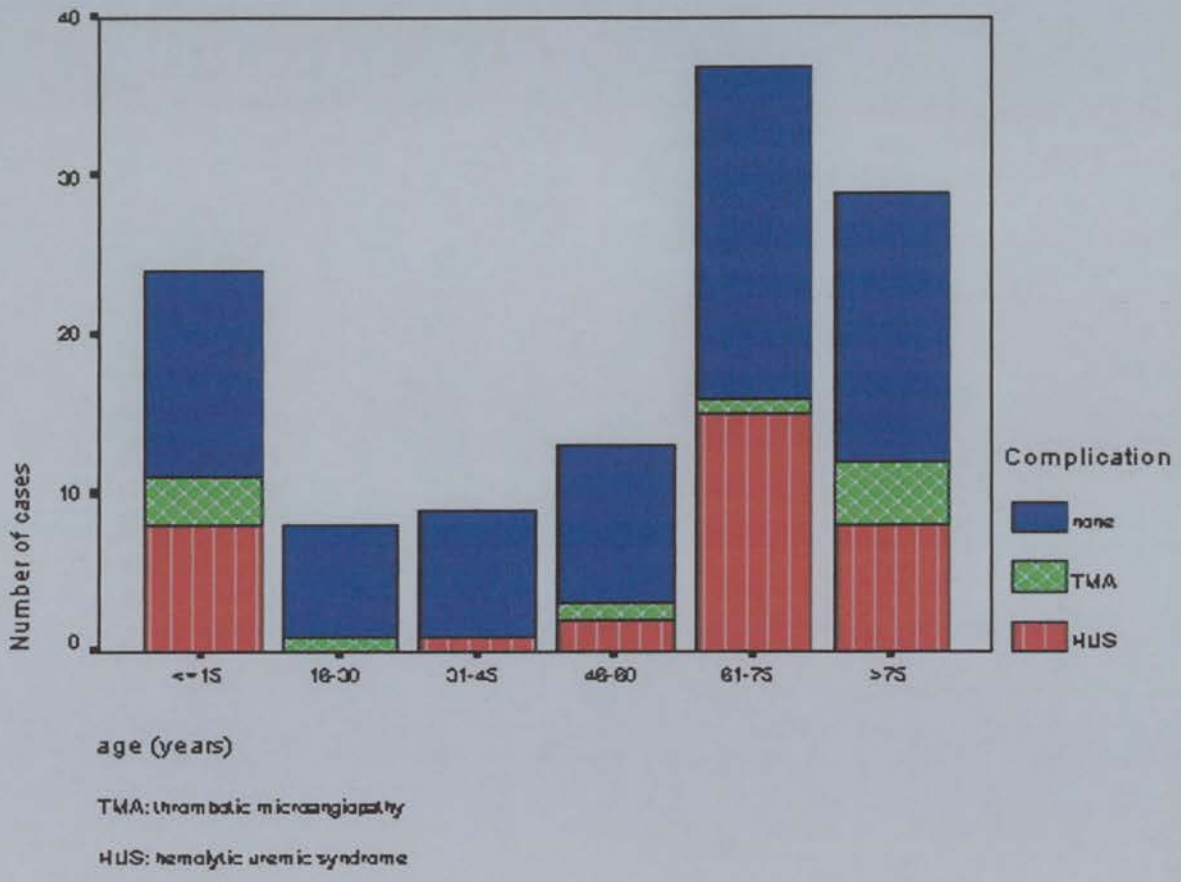
Demographic details and clinical symptoms: Childhood and older age were significantly associated with development of HUS (figure 4.1). 29 of 82 (35%) cases aged < 15 years or > 65 years developed HUS compared to 5 of 38 (13%) cases aged 15 – 65 inclusive (adjusted OR 4.4; 95% CI 1.3-14.4), (Table 4.1). Fever (adjusted OR 2.7; 95% CI 1.0-7.6) and admission tachycardia, (> 100/min adults and >120/min children), (adjusted OR 7.9; 95% CI 1.5-42.4) were associated with progression to HUS. No other gastrointestinal or systemic features predicted development of HUS.

Premorbid medications and chronic illness: 9 of 15 (60%) adults with low gastric acid (previous gastrectomy or taking proton pump inhibitors/ H2 receptor antagonists) developed HUS (adjusted OR 6.7; 95% CI 1.9-24.0), (Table 4.2).

Antibiotics: 8 of 14 (57%) cases treated with any antibiotic in the four weeks prior to the onset of *E. coli* O157 related symptoms developed HUS (adjusted OR 4.7; 95% CI 1.4-16.5), (Table 4.3). 7 of 15 (47%) cases treated with ciprofloxacin within 4 days of symptom onset and 26 of 104 (25%) cases who received no antibiotic treatment developed HUS, but this difference did not reach significance, (OR 2.63; CI 0.76-9.02), (Table 4.1).

Laboratory Investigations: In a univariate analysis, white blood cell count greater than 20 x 10^9/l (OR 8.3; 95% CI 1.1-60.3) and an absolute neutrophil count >15 x 10^9/l (OR 8.5; 95% CI 1.5-50.1), within 48 hours of symptom onset, were both significantly associated with the development of HUS. Serum albumin < 35g/l,

Figure 4.1 Age distribution of cases admitted to hospital who developed microvascular complications of *E. coli* O157



within 48 hours of symptom onset, was also associated with the development of HUS (OR 7.2; 95% CI 1.2-42.5), (Table 4.4).

Sequential analysis of laboratory results for the first 14 days of illness on all 120 admissions included 901 samples, (Figure 4.2). This analysis confirmed that neutrophilia and hypoalbuminaemia precede the laboratory changes regarded to be indicative of the development of HUS. Significant differences in mean neutrophil count, between those who developed HUS and those who did not, were present on day two of their clinical illness. The traditional markers of HUS development (LDH, urea, creatinine and platelet count) became significantly different in the two groups between day four and six of the onset of diarrhoeal symptoms. The sensitivity of a neutrophil count >15 $x10^9$ /l as a predictor of HUS was 94% and the specificity 78%.

4.3.4 Predictors of death

All deaths occurred in patients over 65 years and so analysis in relation to this outcome was confined to this age group. 11 of 16 (69%) of cases who died had HUS and 3 of 16 had TMA. Dehydration and feeling light-headed on admission were the only additional prognostic variables associated with death, (Table 4.1).

4.4 Discussion

This study of hospitalised cases comprised the largest number of adult cases of HUS and deaths ever attributed to *E. coli* O157. It confirmed age (< 15 or > 65 years) to be an important predictor of HUS development. Early neutrophilia also consistently

Table 4. 1: Demographic features, clinical symptoms, signs and antibiotic therapy of 120 cases with E. coli O157, hospitalised during the 1996 central Scotland outbreak: logistic regression analyses used to examine these factors association with Hemolytic Uraemic Syndrome/ Thrombotic Thrombocytopaenic Purpura (HUS/TTP) and death.

Factor		All cases	HUS/TTP			Cases aged over 65 years	Death		
		N (%)	n (% of N)	OR (95% CI)	adjusted OR (95% CI)	N1 (%)	n1 (% of N1)	OR (95% CI)	adjusted‡ OR (95% CI)
Study group		120 (100%)	34 (28%)			58 (100%)	16 (28%)		
Demographic data									
Age (years)	< 15	24 (20%)	8 (33%)	3.61 (1.3-10.1)§	4.35 (1.3-14.4)§	0 (0%)	0 (0%)		
	15-65	38 (32%)	5 (13%)	1.00 (Baseline)	1.00 (Baseline)	0 (0%)	0 (0%)		
	> 65	58 (48%)	21 (34%)	3.61 (1.3-10.1)§	4.35 (1.3-14.4)§	58 (100%)	16 (28%)		
Gender	Male	41 (34%)	12 (29%)	*		18 (31%)	7 (39%)	*	
	Female	79 (66%)	22 (28%)			40 (69%)	9 (23%)		
Clinical symptoms and signs									
Incubation† (< 5 days)	Yes	46 (61%)	16 (35%)	3.47 (1.0-11.7)		24 (65%)	10 (42%)	*	
(missing = 44)	No	30 (39%)	4 (13%)	1.00 (Baseline)		13 (35%)	1 (8%)		
Bloody diarrhoea	Yes	105 (88%)	31 (30%)	*		50 (86%)	16 (32%)	*	
	No	15 (12%)	3 (20%)			8 (14%)	0 (0%)		
Abdominal pain	Yes	102 (85%)	30 (29%)	*		48 (83%)	13 (27%)	*	
	No	18 (15%)	4 (22%)			10 (17%)	3 (30%)		
Fever	Yes	22 (18%)	10 (45%)	2.57 (1.0- 6.7)	2.74 (1.0- 7.6)	11 (19%)	5 (45%)	*	
	No	98 (82%)	24 (24%)	1.00 (Baseline)	1.00 (Baseline)	47 (81%)	11 (23%)		
Vomiting	Yes	59 (49%)	16 (25%)	*		21 (36%)	4 (19%)	*	
	No	61 (51%)	18 (30%)			37 (64%)	12 (32%)		
> 10 stools/day	Yes	51 (43%)	19 (37%)	*		24 (41%)	7 (29%)	*	
	No	69 (47%)	15 (22%)			34 (59%)	9 (26%)		

§ Age groups < 15 years and > 65 years combined in logistic regression analyses for HUS/TTP.
† Data on incubation period not included in multiple logistic regression due to extent of missing data.
* Non-significant association at the 5% level.
‡ Only one variable (i.e. dehydration) remained significant in the multiple regression.
(continued on next page)

Table 4.1 continued

Factor		All cases		HUS/TTP		Cases aged over 65 years		Death	
		N (%)	n (% of N)	OR (95% CI)	adjusted OR (95% CI)	N1 (%)	n1 (% of N1)	OR (95% CI)	adjusted‡ OR (95% CI)
Study group		120 (100%)	34 (28%)			58 (100%)	16 (28%)		
Abdominal tenderness (missing = 1)	Yes	68 (57%)	19 (28%)	*		31 (58%)	10 (32%)	*	
	No	51 (43%)	14 (28%)			27 (42%)	6 (22%)		
Abdominal distension	Yes	10 (8%)	4 (40%)	*		7 (12%)	4 (57%)	*	
	No	110 (92%)	30 (27%)			51 (88%)	12 (24%)		
Tachycardia (missing = 1)	Yes	8 (7%)	6 (75%)	9.33 (1.8-48.9)	7.91 (1.5-42.4)	3 (5%)	1 (33%)	*	
	No	111 (93%)	27 (24%)	1.00 (Baseline)	1.00 (Baseline)	55 (95%)	15 (27%)		
Hypotension (missing = 4)	Yes	4 (3%)	3 (75%)	*		3 (5%)	1 (33%)	*	
	No	112 (97%)	30 (27%)			52 (95%)	14 (27%)		
Dehydration (missing = 1)	Yes	16 (13%)	7 (44%)	*		11 (19%)	6 (55%)	4.44 (1.1-17.6)	4.44 (1.1-17.6)
	No	103 (87%)	26 (25%)			47 (81%)	10 (21%)	1.00 (Baseline)	1.00 (Baseline)
Antibiotic therapy									
Ciprofloxacin (< 4 days from onset) (missing = 1)	Yes	15 (13%)	7 (47%)	*		11 (19%)	5 (45%)	*	
	No	104 (87%)	26 (25%)			47 (81%)	11 (23%)		

* Non-significant association at the 5% level.

Table 4.2: Pre-morbid illness and regular medication of adult cases with E. coli O157, hospitalised during the 1996 central Scotland outbreak: logistic regression analyses used to examine these factors association with Hemolytic Uraemic Syndrome/ Thrombotic Thrombocytopaenic Purpura (HUS/TTP) and death.

Factor		Cases aged over 15 years N (%)	HUS/TTP			Cases aged over 65 years N1 (%)	Death	
			n (% of N)	OR (95% CI)	adjusted† OR (95% CI)		n1 (% of N1)	OR (95% CI)‡
Study group		95 (100%)	25 (26%)			58 (100%)	16 (28%)	
Pre-morbid illness								
Ischaemic heart	Yes	32 (34%)	11 (34%)	*		28 (48%)	9 (32%)	*
disease	No	63 (66%)	14 (22%)			30 (52%)	7 (23%)	
Hypertension	Yes	17 (18%)	5 (29%)	*		15 (26%)	1 (7%)	*
	No	78 (82%)	20 (26%)			43 (74%)	15 (35%)	
Cardiac failure	Yes	5 (5%)	1 (20%)	*		5 (9%)	2 (40%)	*
	No	90 (95%)	24 (27%)			53 (91%)	14 (26%)	
Cerebrovascular	Yes	15 (16%)	5 (33%)	*		12 (21%)	4 (33%)	*
disease	No	80 (84%)	20 (25%)			46 (79%)	12 (26%)	
Diabetes Mellitus	Yes	3 (3%)	2 (67%)	*		1 (2%)	0 (0%)	*
	No	92 (97%)	23 (25%)			57 (98%)	16 (28%)	
Hypochlorhydria	Yes	15 (16%)	9 (60%)	6.00 (1.9-19.3)	6.73 (1.9-24.0)	10 (17%)	6 (60%)	5.70 (1.4-24.1)
	No	80 (85%)	16 (20%)	1.00 (Baseline)	1.00 (Baseline)	48 (83%)	10 (21%)	1.00 (Baseline)
Regular medication								
Aspirin	Yes	18 (19%)	6 (33%)	*		16 (28%)	3 (19%)	*
	No	77 (81%)	19 (25%)			42 (72%)	13 (31%)	
ACE inhibitor	Yes	4 (4%)	1 (25%)	*		4 (7%)	1 (25%)	*
	No	91 (96%)	24 (26%)			54 (93%)	15 (28%)	

* Non-significant association at the 5% level.

† Adjusted for age (i.e. group aged 15-65 years versus group aged > 65 years - see Table 1).

‡ Only one variable (i.e. hypochlorhydria) was significantly associated with outcome of death, and so a multiple regression analysis was not performed.

Table 4.3: Pre-admission treatment of 86 cases with E. coli O157, hospitalised during the 1996 central Scotland outbreak: logistic regression analyses used to examine these factors association with Hemolytic Uraemic Syndrome/ Thrombotic Thrombocytopaenic Purpura (HUS/TTP) and death.

Factor		Cases interviewed N (%)	HUS/TTP n (% of N)	HUS/TTP OR (95% CI)	HUS/TTP adjusted† OR (95% CI)	Cases aged over 65 years N1 (%)	Death n1 (% of N1)	Death OR (95% CI)
Study group		86 (100%)	23 (27%)			42 (100%)	10 (24%)	
Pre-admission treatment								
Antibiotics in the 4	Yes	14 (16%)	8 (57%)	5.07 (1.5-16.8)	4.71 (1.4-16.5)	10 (24%)	4 (40%)	*
weeks before the	No	72 (84%)	15 (21%)	1.00 (Baseline)	1.00 (Baseline)	32 (76%)	6 (19%)	
development of symptoms								
Anti-diarrhoeal	Yes	26 (31%)	9 (35%)	*		16 (38%)	3 (19%)	*
agents	No	60 (70%)	14 (23%)			26 (62%)	7 (27%)	

* Non-significant association at the 5% level.

† Adjusted for age (i.e. group aged 15-65 years versus group aged <15 & > 65 years - see Table 1).

Table 4.4: Laboratory data taken within 48 hours after onset of symptoms: univariate logistic regression analyses used to examine these factors association with Haemolytic Uraemic Syndrome/Thrombotic Thrombocytopaenic Purpura (HUS/TTP) and death.

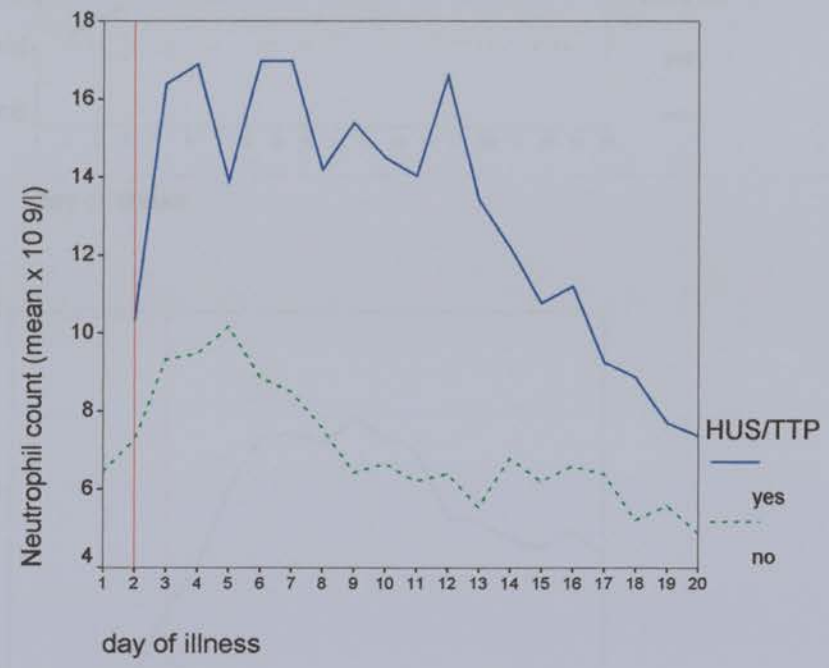
Factor		All cases	HUS/TTP		Cases aged over 65 years	Death	
		N (%)	n (% of N)	OR (95% CI)	N1 (%)	n1 (% of N1)	OR (95% CI)
Study group		44 (100%)	9 (20%)		22 (100%)	6 (27%)	
Neutrophil count	Yes	7 (16%)	4 (57%)	**8.53 (1.5 - 50.1)**	6 (27%)	3 (50%)	*
>15 x 10^9 /1	No	37 (84%)	5 (14%)	1.00 (Baseline)	16 (73%)	3 (19%)	
WCC	Yes	5 (11%)	3 (60%)	**8.25 (1.1 - 60.3)**	5 (23%)	3 (60%)	*
>20 x 10^9 /1	No	39 (89%)	6 (15%)	1.00 (Baseline)	17 (77%)	3 (18%)	
Albumin	Yes	9 (23%)	4 (44%)	**7.20 (1.2 - 42.5)**	9 (47%)	4 (44%)	*
< 35g/l	No	30 (77%)	3 (10%)	1.00 (Baseline)	10 (53%)	2 (20%)	
(missing = 5)							
Creatinine	Yes	6 (14%)	3 (50%)	*	6 (27%)	2 (33%)	*
> 120 umol/l	No	38 (86%)	6 (16%)		16 (73%)	4 (25%)	
Urea	Yes	8 (18%)	3 (38%)	*	8 (36%)	1 (13%)	*
>10 umol/l	No	36 (82%)	6 (17%)		14 (64%)	5 (36%)	
LDH	Yes	1 (3%)	0 (0%)	*	1 (6%)	0 (0%)	*
>600 U/1	No	36 (97%)	7 (19%)		16 (94%)	6 (38%)	
(missing = 7)							
Platelet count	Yes	12 (27%)	3 (25%)	*	10 (45%)	2 (20%)	*
<200 x 10^9/1	No	32 (73%)	6 (19%)		12 (55%)	4 (33%)	
Haemoglobin	Yes	2 (5%)	1 (50%)	*	2 (9%)	1 (50%)	*
<12g/1	No	42 (95%)	8 (19%)		20 (91%)	5 (25%)	
Sodium	Yes	4 (9%)	2 (50%)	*	4 (18%)	2 (50%)	*
	No	40 (90%)	7 (13%)		18 (82%)	4 (22%)	

* Non-significant association at the 5% level.

Figure 4.2

Mean of laboratory results on each day of illness for cases who developed HUS and those who did not

Red vertical reference line: first day of illness on which there is a significant difference in t-test ($p<0.05$)



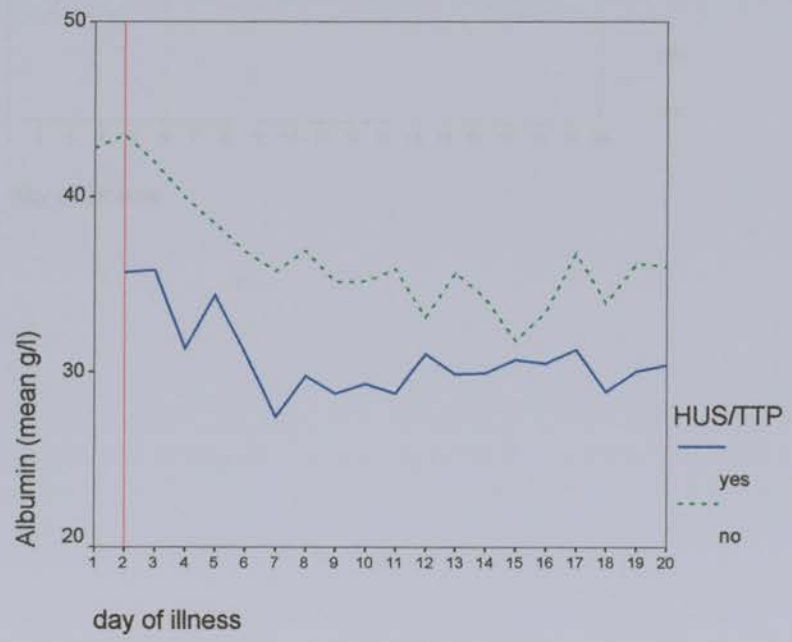
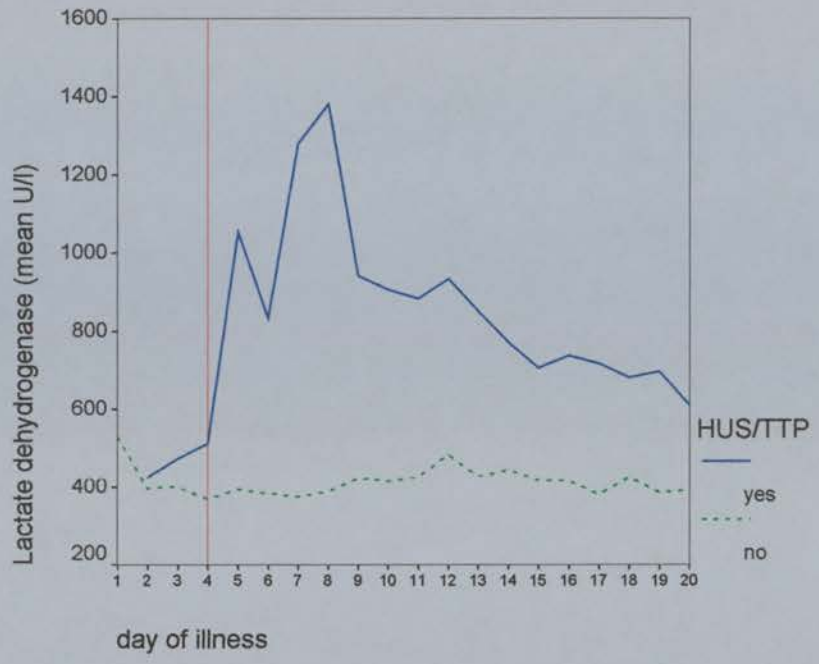


Figure 4.2


Lactate dehydrogenase (mean U/l)
1600
1400
1200
1000
800
600
400
200
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
day of illness
HUS/TTP
yes
no

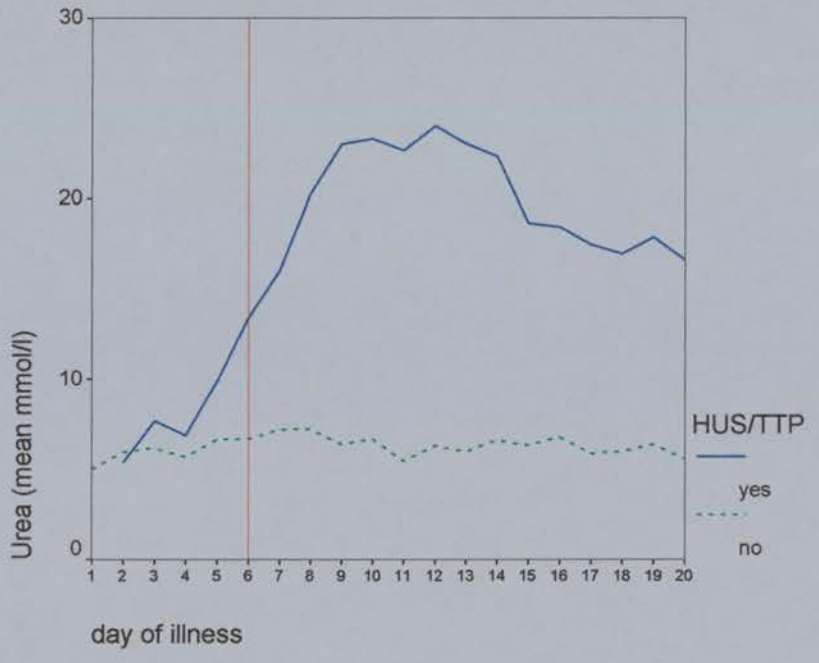
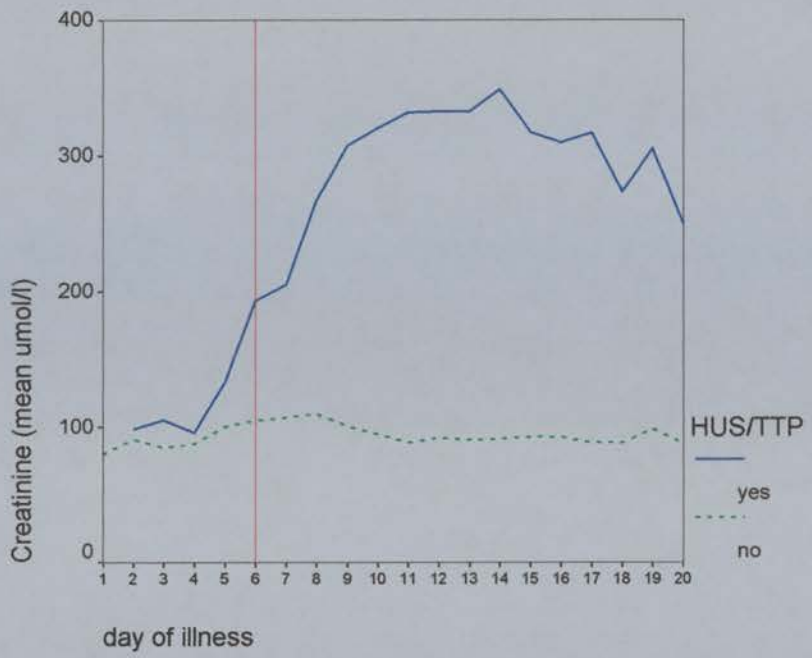

Urea (mean mmol/l)
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
day of illness
HUS/TTP
yes
no

Figure 4.2


400
300
200
100
0
Creatinine (mean umol/l)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
day of illness
HUS/TTP
yes
no

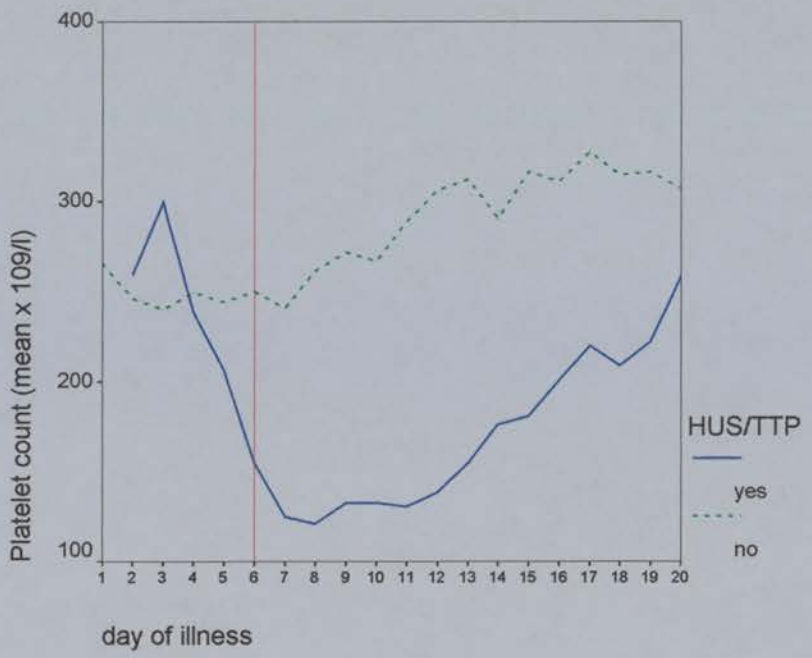
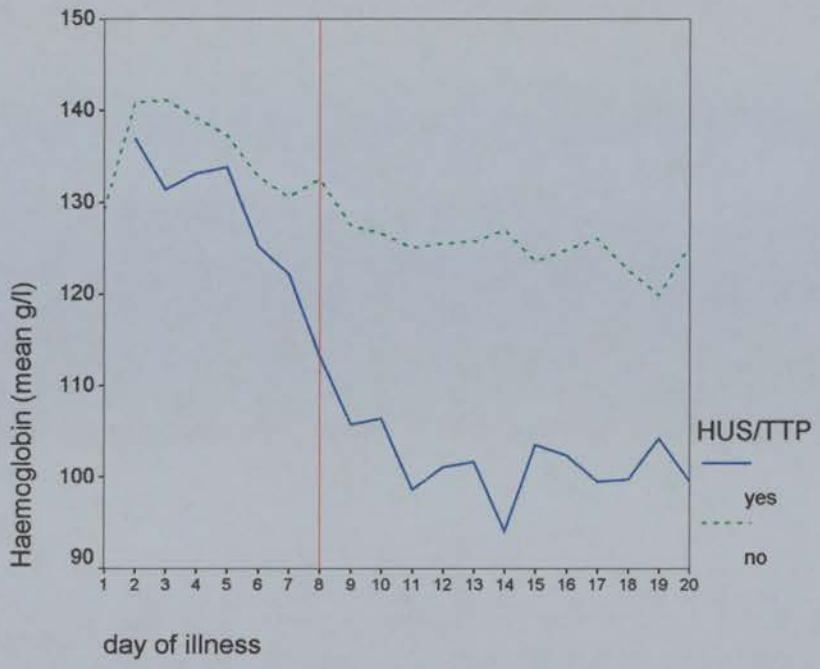

400
300
200
100
Platelet count (mean x 109/l)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
day of illness
HUS/TTP
yes
no

Figure 4.2


150
140
130
120
110
100
90
Haemoglobin (mean g/l)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
day of illness
HUS/TTP
yes
no

predicted the development of HUS and often preceded changes in other laboratory markers by several days. This is in keeping with findings from the study in suspected cases in the community and previous studies in children admitted to hospital. The consistent association of neutrophilia with HUS reinforces speculation that neutrophils are pivotal in the pathogenesis of endothelial injury, speculation which has been validated by the recent discovery that neutrophils transport verotoxin in the circulation [Monnens, 2000].

The finding that hypoalbuminaemia also preceded and predicted HUS is new. The association of hypoalbuminaemia and HUS may be a manifestation of severe gastrointestinal infection or capillary leak secondary to endothelial injury, but may also reflect the fall in albumin seen in older people, in whom it is a well recognised indicator of poor prognosis [Hodkinson, 1981].

Observations from the earliest outbreaks and a controlled clinical trial failed to clarify the role of antibiotics in the development of HUS [Carter *et al.*, 1987, Pavia *et al.*, 1990, Cimolai *et al.*, 1990, Bell *et al.*, 1997, Proulx *et al.*, 1992]. Retrospective analyses of the massive 1996 Sakai City outbreak in Japan contributed to this conflict, children treated early with fosfomycin had reduced incidence of HUS [Takeda *et al.*, 1998, Shioi *et al.*, 1999]. A prospective clinical trial in children in the USA has demonstrated a clear association between sulpha containing and beta lactam antibiotics and increased risk of HUS [Wong *et al.*, 2000]. In vitro evidence has shown 4-quinolones to increase the release of verotoxin [Kimmitt *et al.*, 1999] and that this effect is mediated by phage replication [Zang *et al.*, 2000]. We were unable

to demonstrate a significant association between early treatment with ciprofloxacin and HUS, however only a small number of selected cases received early antibiotic therapy. We did demonstrate an association between antibiotic use preceding *E. coli* O157 related symptoms and the development of HUS. This association had not been previously noted and may be a clinical manifestation of the effect of subtherapeutic antibiotics. In vitro experiments have shown that subtherapeutic or inappropriate antibiotics increase the release of verotoxin [Yoh *et al.*, 1999]. It now seems likely that the effect of antibiotics on verotoxin production is dependant both on their mechanism of action and achieving therapeutic concentrations. Our observations with ciprofloxacin and coincidental antibiotics advocate against the use of antibiotic therapy in *E. coli* O157 infection.

The association of prior gastrectomy/acid lowering drugs with progression from gastrointestinal infection with *E. coli* O157 to HUS, has not been previously noted. Patients have been shown to be at increased susceptibility to other types of bacterial gastroenteritis after gastrectomy [Gianella *et al.*, 1973], and one outbreak suggested that previous gastrectomy was associated with acquiring *E. coli* O157 infection [Carter *et al.*, 1987]. *E. coli* O157 is an acid tolerant organism and hypochlorhydria was not thought to be relevant in its pathogenesis, however there is now evidence of a wide range in acid tolerance among different isolates of *E. coli* O157 [Brudzinski *et al.*, 1998]. If the outbreak strain had reduced acid tolerance more severe illness would be expected in cases with reduced gastric acid.

Fever on admission was found to be associated with HUS in adults as it was in children during the 1996 Japanese outbreak [Ikeda *et al.*, 1999, Honda, 1999]. Fever therefore is likely to be a good surrogate marker for the severity of the inflammatory response.

This detailed retrospective study of cases admitted to hospital investigated predictors of HUS and death within an *E. coli* O157 outbreak with predominantly adult cases and a large number of deaths. The methods of the study ensure that no cases were overlooked, that laboratory results and the collection of clinical data were both accurate and complete. This was however a retrospective study and as such may be limited by the accuracy and completeness of record keeping during the outbreak period.

This study has shown that mortality associated with HUS in adults remains high, even with intensive management. We confirmed that age was the most important risk factor for HUS and that neutrophilia as an early predictor extended to the adult population. Additionally we identified low gastric acid and antibiotics prior to acquiring infection as risk factors. The risk factors identified suggest avenues for further research on the pathogenesis of HUS at a molecular level.

The community clinic and hospital studies provided complementary results. Both have demonstrated risk factors for HUS, which can be defined and will facilitate the management of outbreak situations and individuals in the future.

CHAPTER 5

Effectiveness of Therapeutic Plasma Exchange in the 1996 Lanarkshire *E. coli* O157 Outbreak

5.1 Introduction

E. coli O157 infection has the potential for serious and potentially life threatening local [Griffin *et al.*, 1990, Morris *et al.*, 1991] and systemic complications [Karmali *et al.*, 1985, MMWR, 1986] of which HUS is the most severe. Acute renal failure is integral to the systemic syndrome and studies from children with diarrhoea associated HUS report that 70 percent require dialysis. The outcome in children has improved significantly over the past two decades, largely due to improved dialysis techniques and mortality rates between five and ten per cent are now the standard. The neurological complications of HUS are responsible for much of this residual mortality.

HUS is mediated by the *E. coli* O157 verotoxins [Tesh and O'Brien, 1991]. Verotoxin is transported from the gastrointestinal tract to the sites of endothelial damage by neutrophils [Monnens, 2000]. Neutrophilia has been shown to predict the development of HUS. The community and hospital studies of the central Scotland outbreak have shown that neutrophilia generally precedes HUS by several days. The first manifestation of the syndrome is TMA which is evidenced by red cell haemolysis on laboratory tests. Therefore there is potentially a therapeutic window for intervention prior to established end organ damage.

A general consensus still credits the use of therapeutic plasma exchange (TPE) in adults with idiopathic HUS and in patients of all ages with TTP [Rock *et al.*, 1991, Ruggenenti *et al.*, 1997]. This is controversial in the context of *E. coli* O157

infection, as experience of TPE in the treatment of HUS induced by this organism is limited. The first and largest cohort within the central Scotland outbreak was a group of pensioners who attended a Christmas church lunch. Consequently a large proportion of infected individuals were elderly. The elderly are known to be at particular risk of developing HUS and the expected mortality rate in those treated conservatively is reported at 88% [Carter *et al.*, 1987]. It was therefore decided early in the course of the Lanarkshire outbreak to utilise TPE in the treatment of adults who developed HUS.

5.2 Subjects and methods

5.2.1 Subjects

Clinical data, obtained by reviewing the case notes of all hospitalised cases in the Lanarkshire area, was linked with laboratory and demographic data from the ISD database. All "confirmed" or "probable" cases of *E. coli* O157 infection, identified in the Lanarkshire area during the outbreak period, were included in the assessment and analysis. Adults were defined as patients greater than or equal to 15 years of age.

5.2.2 Methods

This study applied the outbreak definition of HUS. All three criteria for HUS had to be met prior to application of the diagnosis, but not necessarily on the same blood sample. One patient was included as having developed HUS despite a minimum platelet count of 228 (on death). He had bloody diarrhoea, an association with an implicated food source, acute renal failure, the criteria for red cell haemolysis and a falling platelet count.

In the assessment of premorbid illness, past medical histories included as relevant were ischaemic heart disease, cardiac failure, hypertension, cerebrovascular disease, renal disease, diabetes and immunosuppression. Pulmonary oedema was diagnosed on clinical and radiological evidence.

TPE was carried out at three centres using three Cobe Spectra Apheresis Systems and a Baxter Fenwell CS-3000 Plus Cell Separator. Plasma was exchanged with 2-2.4 litres of fresh frozen plasma (FFP) or cryosupernatant (SNBTS) in refractory patients [Rock *et al.*, 1996]. The anticoagulant used was ACD-A. Vascular access was a combination of central and peripheral venous access. Intravenous hydrocortisone was given with each exchange. Intravenous prostacyclin was also given to cases receiving TPE, at doses between 40mg and 200mg/hr, where tolerated.

5.2.3 Statistical Analysis

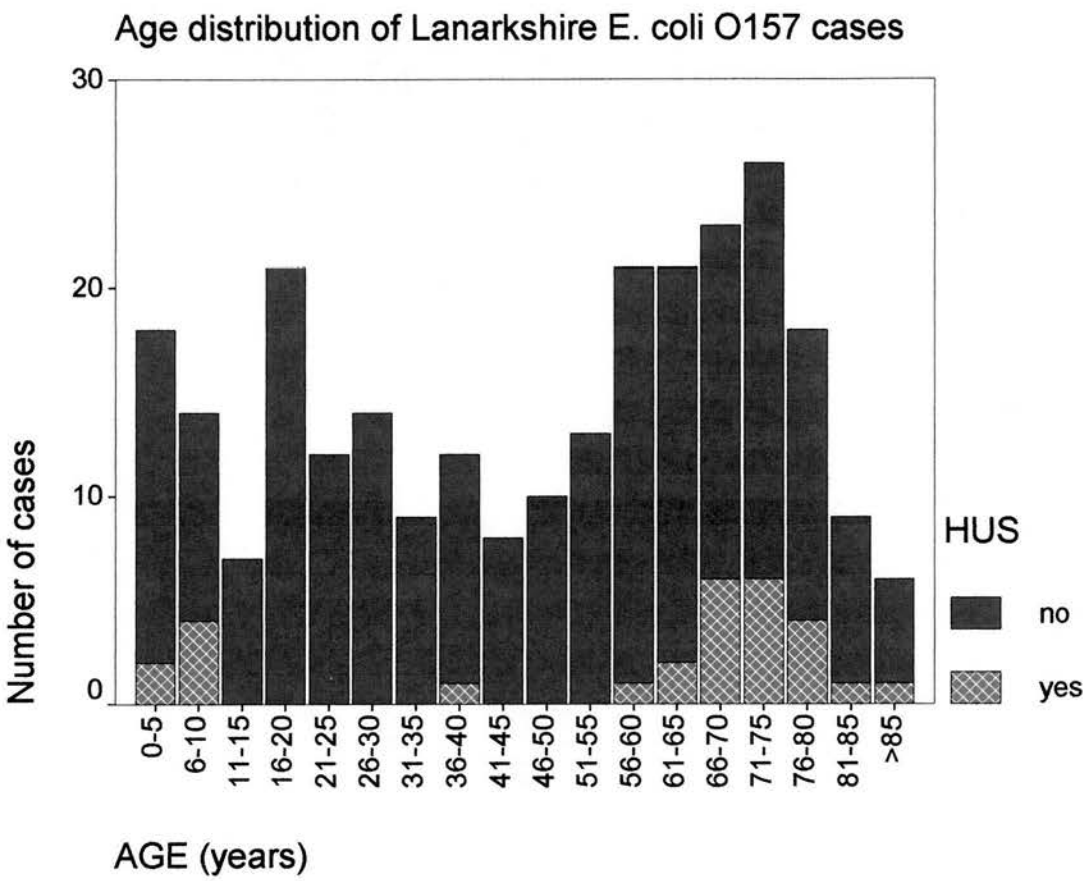
Data was analysed using SPSS version 7.5.

5.3 Results

5.3.1 Patient demographics

There were 262 cases of *E. coli* O157 infection in the Lanarkshire area: 200 confirmed and 62 probable cases. The median age of all affected was 53 years however cases polarised at the extremes of age (Figure 5.1). 47% (124/262) of infected individuals were over 55 years of age. Thirteen (5%) cases died. In 10 cases death was associated with the systemic complications of *E. coli* O157 infection.

Figure 5.1


Age distribution of Lanarkshire E. coli O157 cases
Number of cases
30
20
10
0
0-5
6-10
11-15
16-20
21-25
26-30
31-35
36-40
41-45
46-50
51-55
56-60
61-65
66-70
71-75
76-80
81-85
>85
AGE (years)
HUS
no
yes

5.3.2 Systemic complications of *E coli* O157

Twenty-eight (11%) of the Lanarkshire cases of *E. coli* O157 satisfied the diagnostic criteria for HUS. Cases satisfied the criteria for HUS a median of 7 days (range 4 to 15 days) after the onset of gastrointestinal symptoms. A further eight cases had evidence of thrombotic microangiopathy but did not satisfy the criteria for HUS and were not eligible for TPE. Twenty-two (79%) cases with HUS were adults and six (21%) were children. The median age of adults who developed HUS was 71 years and the median age of children 6 years. The demographics, clinical features, treatment, laboratory results and outcome of the adult cases with HUS are shown in Table 5.1. Blood results are taken from the day that the diagnostic criteria for HUS were met, prior to TPE in cases so treated.

The mortality rate in adults with HUS was 45% (10 of 22). Mortality was 58% (7 of 12) in those aged over 70 years and 30% (3 of 10) in those aged 70 years or less. There were no deaths in children. All cases who died had a post mortem carried out. Causes of death in patients with HUS were acute renal failure secondary to HUS (2 cases), cardiac arrest (2 cases), intracerebral haemorrhage, cerebral infarction, acute myocardial infarction, multiple organ failure, hepatorenal syndrome secondary to macronodular cirrhosis and septic shock.

5.3.3 TPE

TPE was carried out on 16 of the 22 adult patients with HUS. Four patients treated with TPE went on to receive haemodialysis in addition, because of deteriorating renal function. Patients who did not receive TPE were either too unwell to tolerate

the procedure or died before TPE could be carried out. The reasons for not performing TPE are detailed in Table 5.2.

In all 16 cases treated with TPE, the first exchange was first carried out within 24 hours of the criteria for HUS being met. The minimum number of exchanges carried out was one, the maximum 16 and the median six. Patients underwent a total of 107 procedures and 1100 units of FFP were used. Two patients proved refractory to treatment with FFP, after five and six exchanges, but were successfully treated with additional TPE using cryosupernatant as the exchange fluid.

In the TPE treated group the mortality rate was 31%, with 50% (4 of 8) mortality in those aged over 70 years and 12.5% (1 of 8) mortality in those aged less than 70 years or less. Premorbid illness, neurological features, treatment with ciprofloxacin or prostacyclin and the laboratory severity of HUS were not associated with death, however the number of cases were too small to allow statistical conclusion.

5.3.4 Complications of TPE

The most frequent complication associated with plasma exchange was pulmonary oedema. This was diagnosed on clinical and radiological grounds in 11 (69%) cases. Pulmonary oedema was not confined to patients undergoing TPE and occurred in 50% (3 of 6) of HUS cases not treated with TPE. Hypocalcaemia (calcium <2.12 mmol/l) occurred in 15 of 16 patients treated with TPE. Although often severe (minimum serum calcium 1.32 mmol/l) intravenous calcium supplements were given when appropriate and no clinical manifestations of hypocalcaemia were observed.

Table 5.1: Adult HUS cases[a,b]

	Age (years)	sex	TPE	prostacyclin	ciproflox acin	dialysis	CNS features	past medical history	pulmonary oedema	urea (mmol/l)	creatinine (umol/l)	neutrophil count (x109/l)	platelet count (x109/l)	LDH (U/l)	Hb (g/dl)	death
1	40	.	*	*	*	*	.	*	*	12	242	31	150	1049	9	.
2	60	.	*	*	.	*	*	.	*	11	215	25	64	1151	9	.
3	62	.	*	*	.	.	.	*	*	19	141	4	32	1028	11	.
4	63	.	*	*	.	.	.	*	.	15	115	10	170	1065	9	.
5	66	.	*	.	.	*	.	.	.	35	272	14	58	1812	10	.
6	69	M	.	.	*	.	.	*	*	16	187	29	250	1043	11	*
7	69	M	*	*	.	.	*	*	*	12	150	15	95	1052	16	.
8	70	M	.		.	.	*	.	*	19	105	9	145	555	13	*
9	70	.	*	.	.	.	.	.	*	13	162	23	83	939	10	.
10	70	M	*	*	*	*	.	.	*	21	259	12	166	765	14	*
11	71	.	*	*	.	.	.	*	.	14	114	7	166	1153	13	.
12	71	.	*	.	*	.	*	*	*	9	139	22	85	1268	13	.
13	71	.	.	.	*	.	*	.	.	34	268	33	97	1410	11	*
14	72	.	*	.	*	.	*	.	*	20	177	22	29	1971	12	*
15	74	M	*	*	.	.	*	*	*	23	196	9	115	1150	8	.
16	74	M	*	.	.	.	.	*	*	15	219	5	86	1349	12	*
17	76	M	.		.	.	*	.	.	34	116	14	148	676	13	*
18	78	.	*	.	.	.	*	*	*	36	313	30	237	1112	12	*
19	79	.	.		*	.	*	*	.	18	158	13	121	799	14	*
20	80	.	*	*	.	.	.	.	.	12	123	9	125	841	10	.
21	83	.	*	.	*	.	*	*	.	12	193	15	129	1729	11	*
22	90	.	.	.	*	.	*	*	*	33	269	13	121	903	14	.
Total N	22	7	16	9	9	4	12	13	14	22	22	22	22	22	22	10

a. * = yes

b. blood results taken from the day that the criteria for HUS were reached, prior to TPE in cases so treated

Table 5.2: Reasons for patients not receiving TPE

Patient number	Age (years)	Sex	Reason
6	69	M	Cardiac arrest prior to transfer for plasma exchange
8	70	M	Diagnosis not suspected
13	71	F	Cardiac arrest prior to transfer for plasma exchange
17	76	M	Fluid overload, pneumonia, septicaemia
19	79	F	Acute abdomen, dementia, relatives wish
22	90	F	Fluid overload, precarious fluid balance

Hypomagnesaemia (magnesium <0.7 mmol/l) occurred with in 8 patients, intravenous magnesium was given as appropriate and no clinical effects were observed. Other complications associated with TPE were MRSA line infection and extravasation.

5.4 Discussion

Until recently HUS was a rare disease in adults with an estimated frequency of one case per million per year [Petit, 1980]. In 50% of cases it was associated with pregnancy, malignant hypertension, HIV, autoimmune disease, cancer or chemotherapy. The remainder of cases were familial or of unknown aetiology. In 1983 the first association of HUS with *E. coli* O157 infection was made [Karmali, 1985] and the incidence of the disorder has since continued to rise in parallel with the global rise in *E. coli* O157 infections [Armstrong *et al.*, 1996]. Following exposure to *E. coli* O157, between three and seven per cent of all patients progress to overt HUS [Mead *et al.*, 1988], but incident rates are much higher in children and the elderly.

HUS has quite a different course and prognosis in adults compared to children. Children with HUS develop acute renal failure precipitately and the treatment of choice is dialysis, which is initiated when the child becomes oliguric. Most children respond to dialysis and this is evidenced by falling morbidity and death rates [Begue *et al.*, 1998]. In the central Scotland outbreak there were no deaths in children. Adults have a different manifestation of disease and appear to develop neurological or cardiovascular problems before the onset of oliguria. Neurological features have

previously been shown to be associated with increased mortality [Hughes *et al.*, 1991] and neurological and cardiovascular complications of HUS were the most frequent causes of death in the Central Scotland outbreak.

There has been no previously reported experience of the use of TPE in the treatment of adults with HUS caused by *E. coli* O157 infection. The only other reported experience of *E. coli* O157 induced HUS in adults, was from a Canadian nursing home outbreak in 1985. In that outbreak 11 of 12 patients who developed HUS died, compared with 10 of the 22 in the central Scotland outbreak. The patients who developed HUS in the nursing home outbreak were slightly older (mean age 83 years) and probably frailer than our patients. All patients from the nursing home outbreak were admitted to hospital and treated with full supportive therapy but without TPE. We believe that our use of TPE was the only treatment difference between our patients and those reported from the Canadian nursing home outbreak. Also in our patients we can find no evidence of any other treatment influencing outcome from HUS. We therefore suggest that without the use of TPE the proportion of HUS cases who died would have been higher.

When HUS develops secondary to gastrointestinal infection with *E. coli* O157 endothelial damage is mediated by the lysogenic-phage encoded verotoxin 1 and 2 [McBrien *et al.*, 1983, Tesh and O'Brien, 1991]. The verotoxins enter the systemic circulation causing microvascular damage at target organs by a process that is thought to be mediated by pro-inflammatory cytokines such as TNF-α and interleukins [Lousi and Obrig, 1991]. Endothelial damage induces the formation of

large von Willebrand factor multimers [Moake and McPherson, 1989], which in turn may cause platelet aggregation with the formation of small vessel thrombi in target organs. These combined processes are manifest clinically as thrombocytopenia, hemolytic anaemia, renal impairment and neurological problems such as confusion, seizures and cerebrovascular accidents. The theoretical basis for use of TPE in *E. coli* O157 infection is that it may remove verotoxins, pro-inflammatory cytokines and von Willebrand factor multimers from the circulation, thereby removing the factors which initiate and perpetuate the microvascular process that leads to HUS.

Plasma exchange is an expensive (£2500 per person treated in our hospital) and intensive procedure. Also it is not without risk and most reported complications relate to the need for central venous catheterisation. The effectiveness of TPE in the treatment of *E. coli* O157 induced HUS needs to be determined definitively. This would best be achieved by a multicentre randomised-controlled trial. When faced with a disease with a very high mortality and only one potentially beneficial treatment option a trial that withholds this option would be difficult to justify. In the context of sporadic and outbreak cases of *E. coli* O157 it would also be extremely difficult to organise logistically. There will always be an unavoidable selection bias within such a trial, with patients who are excluded from treatment because they have contraindications to TPE or who die before treatment can be initiated.

Assuming five per cent of all cases of *E. coli* O157 develop HUS approximately 40 adult cases of HUS per year would be expected in the UK (data from PHLS Communicable Disease Surveillance Centre and SCIEH). A national register should

be established for adult cases of HUS, as was recently operative for paediatric cases. This database would enable monitoring of treatment and outcomes in adults, providing definitive evidence of the effectiveness of TPE within about 5 years.

In the interim, we have reported our use of TPE in the treatment of the largest number of adult cases of HUS secondary to *E. coli* O157. We have recorded an overall mortality rate of 45%, compared with a historical mortality rate of 88%. We believe this provides evidence for the effectiveness of TPE in the treatment of adults with HUS caused by *E. coli* O157. There is no evidence from our experience that TPE is harmful. A national register of HUS secondary to *E. coli* O157 could define the role of TPE in the treatment of this serious condition.

CHAPTER 6

Blood Group and Susceptibility to Disease Caused by *E. coli* O157

6.1 Introduction

Morbidity and mortality associated with the 1996 outbreak of *E. coli* O157 in central Scotland raises the question of why some individuals exposed to this organism developed severe disease and others did not. Aside from the extremes of age, few demographic factors have been associated with susceptibility to this disease. Studies of other enteric pathogens indicate blood group is a risk factor for some of these diseases. Occurrence or severity of diarrhoeal disease due to *E. coli* [Black *et al.*, 1987], *Vibrio cholerae* and *Helicobacter pylori* are associated with two genetic characteristics: the O blood group; and the inability of individuals to secrete the water-soluble glycoprotein forms of their respective ABO blood group antigens (non-secretion) [Swerdlow *et al.*, 1994, Alkout *et al.*, 1997, Chaudhuri and Das Adhikary, 1978]. The P blood group is a receptor on endothelial surfaces for the verotoxin [Bitzan M *et al.*, 1994], but there have been conflicting reports on association between P blood group and susceptibility to disease due to *E. coli* O157 in Caucasian populations [Taylor *et al.*, 1990, Robson *et al.*, 1994, Ashida *et al.*, 1999].

Previous work demonstrated that *H. pylori* bound in higher numbers to cells of individuals of blood group O and binding of the bacteria was significantly associated with expression of H type 2, the antigen of blood group O [Alkout *et al.*, 1997]. In addition, individuals of group O produced significantly higher levels of tumor necrosis factor α (TNF) and interleukin 6 (IL-6) in response to *H. pylori* [Alkout *et al.*, 2000].

The aims of the study were:

1) to assess ABO, P blood groups and secretor status among patients with disease due to *E. coli* O157 in comparison with the population in central Scotland.
2) to determine if the H type 2 antigen is a receptor for the outbreak strain or other strains of *E. coli* O157.
3) to assess inflammatory responses to a culture filtrate of the outbreak strain in relation to ABO and P blood groups.

6.2 Subjects and Methods

6.2.1 Subjects

There were 186 patients in the study who developed disease due to confirmed *E. coli* O157 infection during the Lanarkshire outbreak. For each case ten possible age and sex matched control subjects were generated from the Lanarkshire CHI by computer selection and with their general practitioners consent they were invited to participate, 122 agreed and are included as control subjects. Blood was taken from cases at the time of infection and from cases and control subjects annually to the third anniversary of infection. Information on ABO but not Lewis or P blood groups was available for 16 individuals who died in Lanarkshire hospitals, 12 from Monklands District General Hospital in Airdrie and 4 from Law Hospital in Carluke. Results for patients and CHI controls were also compared with figures obtained for previous studies in central Scotland [Blackwell *et al.*, 1990, Peebles Brown *et al.*, 1994].

Patients with clear evidence of red cell haemolysis were regarded as having VT-mediated TMA, a proportion of which progressed to HUS. HUS was defined by the original outbreak criteria.

6.2.2 Determination of blood groups

ABO group was determined by slide agglutination with monoclonal anti-A and anti-B reagents from the Scottish National Blood Transfusion Service (SNBTS). Lewis antigens were determined by tube agglutination with monoclonal anti-Lewis^a and anti-Lewis^b (SNBTS). Polyclonal anti-P typing serum (SNBTS) was used in slide agglutinations and results recorded as 3 = strong, 2 = moderate, 1 = weak and 0 = no visible agglutination. The cells were also incubated with the anti-P serum for 30 min at room temperature and agglutination assessed by flow cytometry in comparison with cells incubated for 30 min with phosphate buffered saline (PBS). Secretor status was assessed by haemagglutination inhibition tests and results compared with results for Lewis^a and Lewis^b antigens by ELISA for Lewis antigens in saliva.

6.2.3 Bacteria

In addition to the outbreak strain, eight additional strains of *E. coli* O157 of different phage types were obtained from Dr. M.F. Hanson, Central Microbiological Laboratory, Western General Hospital, Edinburgh. The bacteria were cultured in category 3 containment facilities and formalin fixed cultures used in the binding assays.

6.2.4 Bacterial binding to epithelial cells

The flow cytometry method described previously was used for assessment of bacterial binding to buccal epithelial cells of the different ABO blood groups and the Kato III gastric epithelial cell line [Alkout *et al.*, 1997]. Blood group antigens were detected with monoclonal antibodies to H type 2, A, B, Lewis^a and Lewis^b by flow cytometry. Bacterial binding was examined in relation to level of expression of blood group antigens reflected in the level of monoclonal antibodies bound [Alkout *et al.*, 1997].

6.2.5 Screening for bacterial adhesins that bind blood group antigens

The spectrophotometric methods for detection of direct binding of biotinylated synthetic blood group oligosaccharides (Syntesome) was used [Alkout *et al.*, 1997]. Binding of fluorescein-labelled blood group oligosaccharides (Syntesome) to the bacteria was also examined by flow cytometry. The strain of *H. pylori* NCTC 11637 [Alkout *et al.*, 1997] which expresses fucose-binding adhesins was included in each experiment as a positive control.

6.2.6 Inflammatory responses to *E. coli* O157 antigens

Inflammatory responses elicited from leukocytes by a sterile culture filtrate of *E. coli* O157 were assessed by blood group and P antigen level of the donor. The bacteria were grown in nutrient broth for 24 h at 37°C in universal containers. The bacteria and filtrates were prepared in a category III containment facility. Buffy coats from blood donors (n = 32) were obtained from the Blood Transfusion Service, Royal Infirmary of Edinburgh. There were 8 sets of A, B, O and AB

donors and the levels of IL-6 and TNF were assessed by ELISA and bioassay respectively [Gordon *et al.*, 1999].

6.2.7 Statistical analyses

Samples were coded so that the blood groups were determined without reference to patient or control group. Results were entered into a database and assessed by the Epi Info package. Prevalence was compared between groups by chi-squared test with Yates' correction and confidence intervals for odds ratios were calculated by exact methods.

6.3 Results

6.3.1 Epidemiological study

ABO and P blood groups were determined for 186 patients from the Lanarkshire outbreak and 122 controls obtained form the CHI list. Because the proportion of group O among the 122 CHI group was higher than that for the population covered by the Lanarkshire Blood Transfusion Service based at Law Hospital (51%, personal communication Dr. K. Liddle), data for ABO groups were also compared with those from our previous study on an outbreak of meningococcal disease at the Airdrie Academy [Blackwell *et al.*, 1990] and data for blood donors in greater Glasgow [Peebles Brown *et al.*,1994]

There was no significant difference between the proportion of group O between patients (63.4%) and the CHI group (58.3%); however compared with the larger

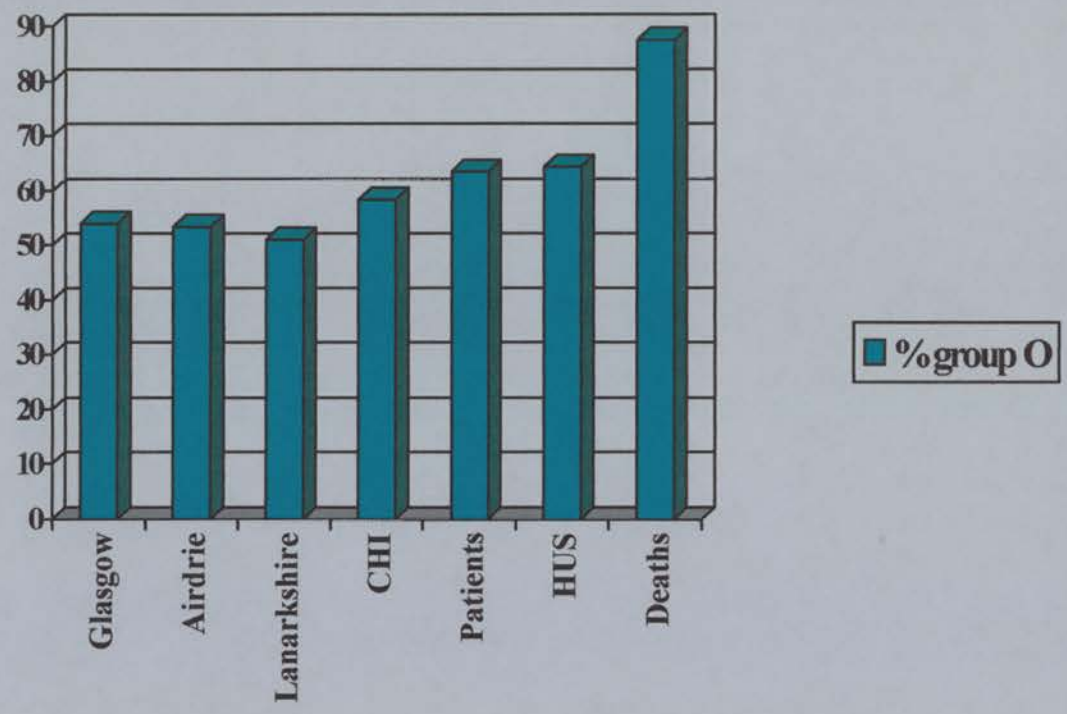
groups, Airdrie (n = 371) (53.4%, $P < 0.05$) and Glasgow (n = 5898) (53.9%, $P < 0.01$), there was a significant increase in group O among the patients. The proportion of patients with HUS who were group O was 18/28 (64.3%); however, among patients who died 14/16 (87.5%) were group O, and this was significantly increased compared to all three control groups ($P < 0.05$) (Figure 6.1). There were no differences in the proportions of secretors and non- secretors of ABO blood group antigens observed between patient and control groups.

Red blood cells from 83 of 135 patients (61.5%) who attended follow up clinics were not agglutinated or only weakly agglutinated by the anti-P antiserum compared with 46 of 113 (40.7%) of the samples from the CHI controls (P = 0.0017, CI 1.35,4.00). Among 17 patients who developed TMA and who attended the follow up clinics, 14 (82.4%) had no or weak reactions with anti-P compared with the CHI controls (P = 0.0032, CI 1.73-38.41). Among patients with TMA, 12 developed HUS and 10 (83.3%) had no or weak reactions with anti-P compared with the CHI controls (P = 0.0130, CI 1.43,70.37). Data on P blood group were not available for patients who died.

6.3.2 Bacterial binding in relation to blood group and secretor status

Nine strains of *E. coli* O157, including the outbreak strain, were tested for binding to epithelial cells of secretor and non-secretor donors of blood groups A, B, O and AB. There was no correlation between binding of the bacteria to the Kato III cell line or epithelial cells from the donors and expression of ABO or Lewis blood group antigens.

Figure 6.1: Percentage of O blood group in cases and different settings central Scotland



6.3.3 Binding of labelled blood group oligosaccharides to *E. coli* O 157

None of the strains bound any of the biotinylated or fluorescein-labelled ABO or Lewis blood group antigens although the oligosaccharides bound to the H. *pylori* control in the assays.

6.3.4 Inflammatory responses and blood group

There were no significant differences in levels of either TNF or IL-6 associated with ABO group. With respect to P antigen on the red cells, there were no differences in IL-6 levels. There were 8 donors whose red cells were not agglutinated by anti-P, and of these, 5 produced TNF levels > 100 IU ml^{-1} in response to the culture filtrate. None of the samples from donors expressing P antigen on their red cells produced TNF responses approaching 100 IU ml^{-1} (P= 0.001).

6.4 Discussion

Among patients affected during the Lanarkshire outbreak of *E. coli* O157, the proportion of group O was 63.4% which was significantly higher than the general populations in the surrounding area (51-53.3%) but not significantly increased compared with the internal control group of 122 individuals extracted from the CHI. The proportion of O individuals who died during the outbreak was significantly increased compared with all three control groups. In contrast to previous studies on *H. pylori* [Alkout *et al.*, 1997, Alkout *et al.*, 2000], increased susceptibility of group O to disease due to *E. coli* O157 was not associated with

increased binding of bacteria to epithelial cells of group O or to higher inflammatory responses of group O leukocytes.

The high proportion of patients with disease due to *E. coli* O157 (61.5%), particularly those who developed TMA (82.4%) or HUS (83.3%), whose erythrocytes were not agglutinated or weakly agglutinated by anti-P antiserum supported the findings obtained for 32 children with HUS. Among these children there was a significant excess of patients with weak of absent expression of P1, particularly among those with poor outcome [Taylor *et al.*, 1990]. Unfortunately there was no information on the P blood group for patients who died. Two possibilities are suggested: 1) the P antigen on erythrocytes can absorb the VT and prevent it reaching target cells in the kidney or brain; 2) VT and/or endotoxin in the culture filtrate is better able to bind to leukocytes of individuals who do not express P or have low levels of this antigen on their cells.

TNF has been implicated as a major factor in tissue damage in an animal model of *E. coli* O157 [Isogal *et al.*, 1998]. High levels of TNF, IL-6, interleukin-8, interleukin 10 and interleukin 1 receptor antagonist (IL-1Ra) have been reported among patients infected with *E. coli* O157 [Proulx *et al.*, 1998]. The induction of very high levels of TNF by the culture filtrate from leukocytes of P-negative individuals is an observation to be pursued. The results of these studies complement findings of an increased proportion of individuals with low levels of P antigen on their red cells in the patient group, particularly among the patients who developed HUS. This observation might help identify individuals at greater

risk of more serious disease caused by *E. coli* O157, in particular those who would benefit from TPE.

This study identified two genetic risk factors for disease due to *E. coli* O157. Although the original hypothesis that individuals of group O might be at increased risk of disease was supported by the data, the variations in levels of the P blood group antigen appear to be more important in relation to severity of disease. These findings provide new avenues to investigate the differences in host susceptibility factors and responses that contribute to development of serious kidney and vascular damage in response to these infections.

CHAPTER 7

Chronic Renal Disease after Gastrointestinal Infection with *E. coli* O157

7.1 Introduction

In the two decades since the systemic complications of *E. coli* O157 were recognised [Karmali *et al.*, 1983], acute mortality in children has improved dramatically and is reported at less than 10% in most centers. Approximately 50% of children with HUS require dialysis [Mead and Griffin, 1988] and improved outcome reflects advances in dialysis techniques. Reported mortality in adults remains much higher. The only two significant adult cohorts of *E. coli* O157 associated HUS are from a Canadian nursing home where the reported mortality rate was 88% in 12 cases [Carter *et al.*, 1987] and from our experience in Central Scotland where the mortality was 42% in 26 cases. It is not clear if the excess residual mortality in adults is a manifestation of advanced age or if it reflects more severe microvascular disease.

The pathological basis of HUS is small vessel wall thickening, intraluminal platelet thrombosis and partial or complete obstruction of the vessel. Global glomerulosclerosis, tubular atrophy and interstitial scarring have been demonstrated in renal biopsies in the convalescent and chronic phase [Moghal *et al.*, 1998]. Therefore the potential for chronic renal disease is evident and the legacy of *E. coli* O157 is becoming apparent. HUS is now the commonest cause of chronic renal failure in children and accounts for 2.7% of children undergoing renal transplant in North America [Warady *et al.*, 1997]. End Stage Renal Failure (ESRF) is the extremity of a spectrum of chronic renal abnormality induced by HUS. The range of renal disease has been extensively investigated in children (Table 7.1, Figure 7.1). The results of published reports when taken as a whole suggest, of the patients who

survive the acute illness, 73% recover normal renal function, 12% have minor abnormalities of renal function of uncertain significance at the time of reporting, 12% have significant abnormality of renal function and 3% develop ESRF.

In adults HUS develops almost exclusively in patients over 60 years of age. The reason for this predilection is not known but it does not appear to reflect premorbid illness. The assessment of impaired renal function in adults after HUS is complicated by the decline in renal function with age (GFR is maintained at approximately 140ml/min/1.73 m2 until the age of 30, thereafter it declines by about 8ml/min/1.73m2 per decade. [Rowe *et al.*, 1976]), comorbidity and concomitant medications, each of which may have an effect on renal function. The large number of adult HUS cases in the central Scotland outbreak of *E. coli* O157 provided opportunity to investigate the chronic renal sequelae of HUS in adults. To address the complexity of assessment of renal function in older age the study included a control group.

7.2 Subjects and methods

7.2.1 Aims

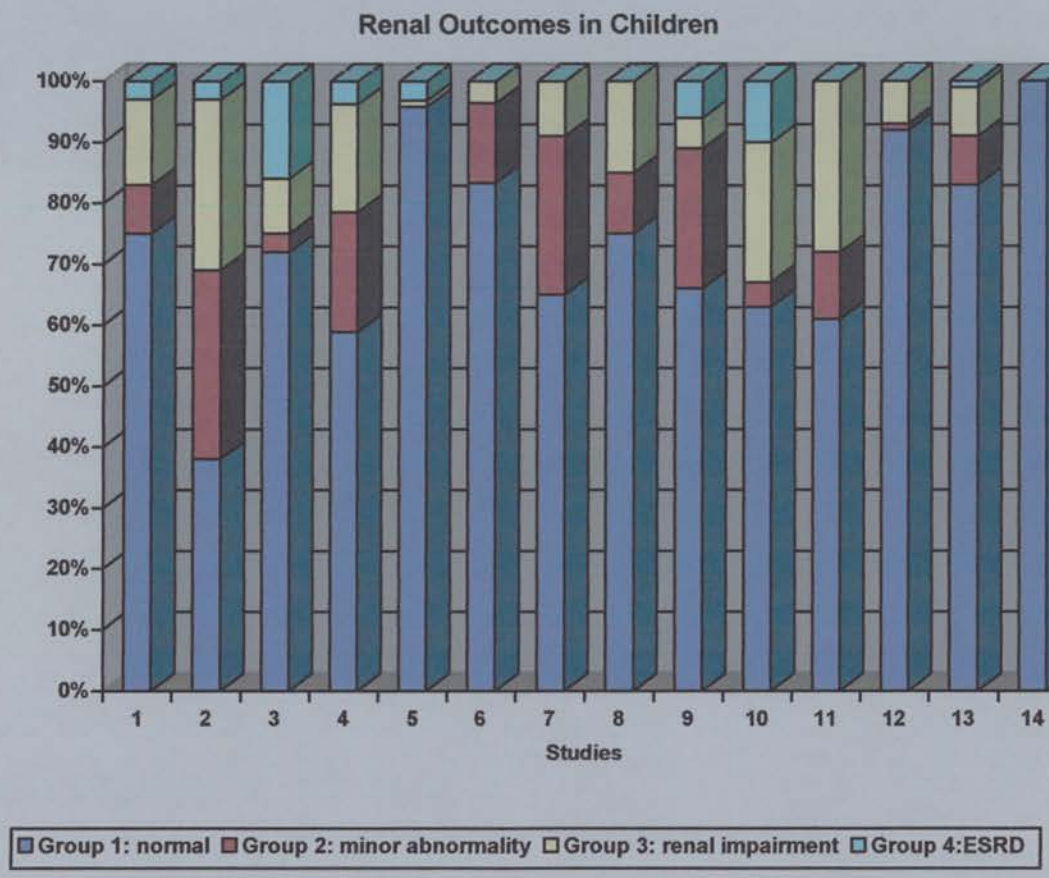
The primary aim was to determine the prevalence of renal disease after HUS, in adults to the third anniversary of infection with *E. coli* O157. A secondary aim was to compare the prevalence of chronic renal disease in adults with published reports

Table 7.1: Published follow up studies of renal disease after HUS

	Reference	Period	Total (D+)	Acute mortality	Dialysis	Age (years)	Follow-up duration (years)	Follow up number	Group 1 (normal)	Group 2 (minor)	Group 3 (impaired)	Group 4 (ESRD)
1	De Jong 1988	1965-1977	96	16 (17%)			10	73	55 (75%)	6 (8%)	10 (14%)	2 (3%)
2	Fitzpatrick 1991	1966-1985	103 (103)	5 (5%)	88	11.6	8.5	88	33 (38%)	27 (31%)	25 (28%)	3 (3%)
3	Trompeter 1983	1969-1980	72 (52)	8 (11%)	57	3.5		69	50 (72%)	2 (3%)	6 (9%)	11 (16%)
4	Spizzirri 1997	1968-1984	312	8 (3%)	70	1.1	13	118	74 (637%)	21 (18%)	19 (16%)	4 (3.4%)
5	Binda 1981	1970-1976	45 (39)	3 (7%)	23	0.25- 14	5	42	38 (91%)	0	1 (2%)	3 (7%)
6	Van Dyck 1988	1970-1976	54 (42)	6 (11%)		0.3- 12	10	46	32 (70%)	11 (24%)	3 (6%)	0
7	Kelles 1994	1970-1982	95 (83)	6 (6%)		1.5	10	80	52 (65%)	21 (26%)	7 (9%)	0
8	Hughes 1991	1972-1988	79 (71)	7 (9%)	59	2.7	4	59	44 (75%)	6 (10%)	9 (15%)	0
9	Loirat 1984	1974-1981	67	5 (7%)	48	3.3	3	56	37 (66%)	13 (23%)	3 (5.5%)	3 (5.5%)
10	Tonshoff 1994	1980-1988	53	2 (4%)	(69%)	3.1	17	48	30 (63%)	2 (4%)	11(23%)	5 (10%)
11	Siegler 1991	< 1983	118	8 (7%)		11.9	9.6	61	37 (61%)	7 (11%)	17 (28%)	0
12	Milford 1990	1985-1988	272	17 (6%)	157	3.5	1	252	233 (92%)	2 (1%)	17 (7%)	0
13	Small G 1998	1986-1996	114 (114)	2 (2%)	82	3	5	40	33 (83%)	3 (8%)	3 (8%)	1 (1%)
14	Yoshioka 1999	1996	15	0	5	7	2	15	15 (100%)	0	0	0
	TOTAL		**1495**	**93 (6%)**				**1047**	**763 (73%)**	**121 (12%)**	**131 (12%)**	**32 (3%)**

Mean/ median or ranges are given as presented in original papers

Figure 7.1 Published studies reporting outcomes from HUS in children



in children. The final aim was to investigate abnormality of renal function in cases with no obvious microvascular complication during the acute illness.

7.2.2 Subjects

Subjects were cases from Lanarkshire infected with *E. coli* O157 during the 1996 central Scotland outbreak, who agreed on the first anniversary of infection to participate in the project. The cases were split into two groups, adults and children (< 15 years). The two groups were further subdivided into cases who had HUS or TMA and cases with uncomplicated gastrointestinal infection. HUS and TMA were defined from the outbreak presentation.

Data analysis on the first anniversary highlighted a wide variation in creatinine clearance (Ccr) measurement without substantiating evidence of renal function abnormality. Therefore on the second and third anniversaries of infection, for adult cases age and sex matched control subjects were sought from the CHI. Ten control subjects for each case were invited to take part.

7.2.3 Methods

Baseline information, from the medical records, was obtained for all cases on premorbid illness, medication and the clinical history of acute infection with *E. coli* O157.

Cases were reviewed at a community clinic annually to the third anniversary; controls were reviewed on the second and third anniversaries. At annual review

participants had blood was taken for measurement of creatinine, submitted a 24hr urine collection for Ccr measurement and timed albumin measurement and an Early Morning Urine (EMU) specimen for microalbumin measurement. Explanation and written instructions were given on the procedure for urine collection. Weight and blood pressure (systolic blood pressure (SBP) and diastolic blood pressure (DBP)) were recorded by standardised method. In children height was measured. New events and medications were documented.

The design was a case observational study for children and adults to the first anniversary and a case control study for adults on the second and third anniversaries. Ccr and blood pressure (BP) in adult cases with HUS were compared with measurements in all control subjects. All adult cases with uncomplicated gastrointestinal infection were compared with a single age and sex matched control. Renal abnormalities persisting in children after the incident were determined and any found were assumed attributable to the *E. coli* O157 infection. Annual review of all participants took place between November and December 1998 and 1999. Ethical approval was granted by Lanarkshire Health Board Research Ethics Committee.

7.2.4 Outcome measures

Four evolution patterns were defined at the end of follow-up:

- Group 1. Complete recovery; (Ccr>80ml/min/ 1.73 m^2, normal BP and no proteinuria).
- Group 2. Minor abnormaility; (Ccr>80ml/min/ 1.73m^2, protein in EMU or 24hr collection

>20 and <250mmol/l, hypertension SBP>160mmHg or DBP>90mmHg).

- Group 3. Renal insufficiency; (Ccr 50-80ml/min/ 1.73m^2 with or without proteinuria > 20mmol/l or hypertension)
- Group 4: Chronic renal failure (Ccr 10-49ml/min/1.73m2 with/without proteinuria or hypertension).
- Group 5. End stage renal failure (ESRF); (Ccr<10ml/min/1.73 m^2 with/without proteinuria or hypertension).

7.2.5 Statistical Analysis

Data was analysed using SPSS version 9. Statistical differences were examined by chi-squared test and two-sample Student's test. $P<0.05$ was considered to be statistically significant and OR>1 taken to indicate association.

7.3 Results

166 (141 adults) cases infected with *E. coli* O157 attended on the first anniversary of infection. 138 (120 adults) cases attended on the second anniversary, and 116 (105 adults) on the third anniversary. 133 control subjects were recruited from the CHI of which 110 attended on the third anniversary.

During the outbreak 28 cases developed HUS and nine TMA. 12 of the 37 cases with a microvascular complication died during the acute illness and one case died 4 months afterwards (Figure 7.2). Of the 24 surviving cases, 15 were adults and nine children. All 15 adults and 4 of 9 children are included in the study (Table 7.2).

Three adults and one child were treated with dialysis, and 11 adults were treated with TPE during the acute illness. Five children who are reviewed annually by the paediatric nephrologists, declined to participate and details of their renal function was obtained from their physician.

7.3.1 Outcome in adults

In 12 adults who had HUS, 1 progressed to ESRD, 3 developed CRF, 8 had clear evidence of renal insufficiency, and none had normal renal function. Of the 3 adults with TMA, one had CRF on a background of pre-existing essential hypertension, and two normal renal function (Table 7.2).

To determine if the measured renal abnormalities in adults could be attributed to HUS or simply reflect age and the limitations of 24 hr urine collection as an estimate of glomerular filtration, all Ccr measurements in HUS cases were compared with all measurements in control subjects. To allow equivalence for age only controls greater than 62 years of age were included in the analysis. There were 33 Ccr measurements in HUS cases and 126 in control subjects over 62 years of age. The mean age of both groups was 71 years. The median Ccr after HUS was 49 umol/l compared to 76 umol/l in controls. There was a significant difference between the mean Ccr after HUS (49umol/l) compared to controls (76umol/l) ($p<0.001$). Ccr <80ml/min/1.73m2 occurred in 31/33 (94%) HUS measurements and 79/126 (63%) control measurements, $p<0.001$, OR 9.2 (2.1-40.3). Ccr <25 ml/min/1.73m2 occurred in 6/33 (18%) HUS and 3/126 (2%) control measurements, $p=0.003$, OR 9.1 (2.1-38.7).

Figure 7.2:
Outcome of microvascular complications of *E. coli* O157 in adults and children infected during the central Scotland outbreak

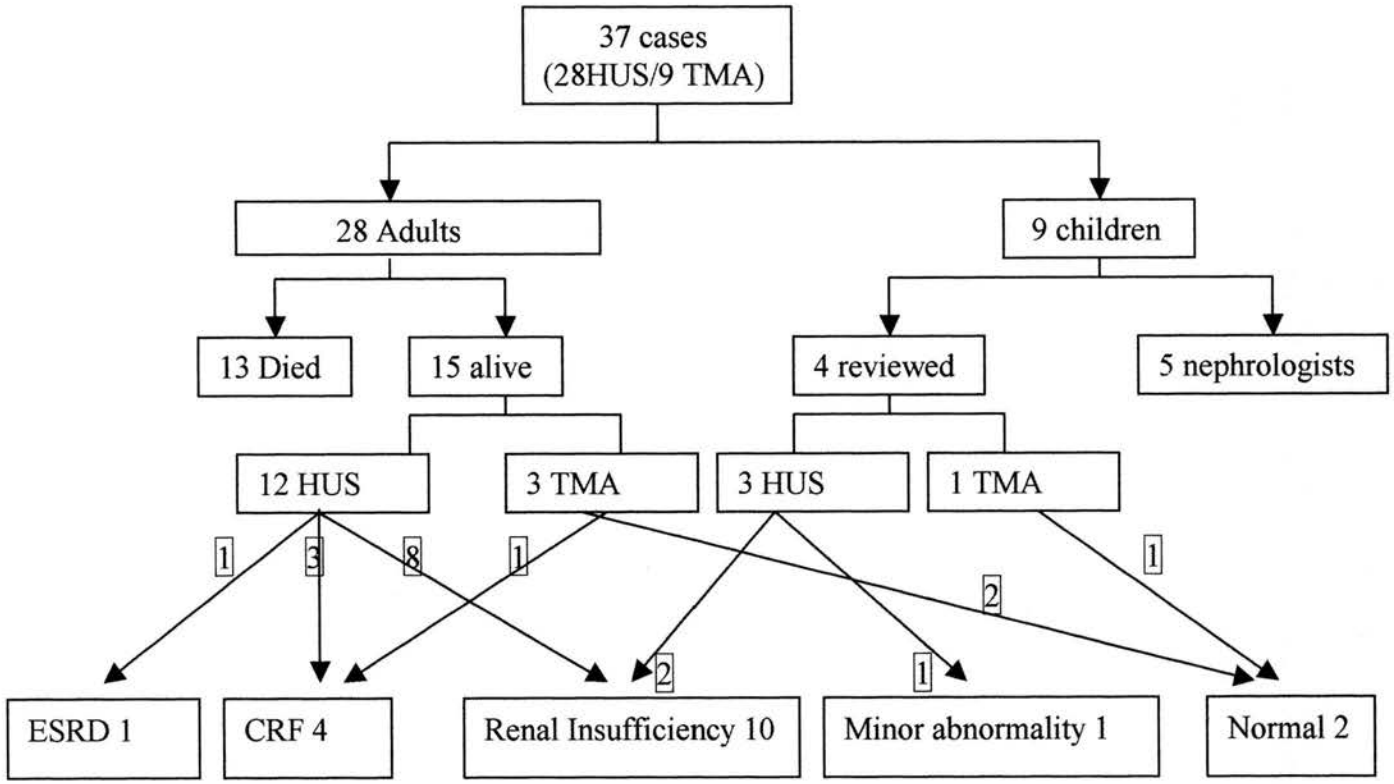


Table 7.2 HUS and TMA cases: Renal function to the third anniversary of infection

	HUS/ TMA	Age	Sex	plasma cr 1/2/3 (umol/l)	Crcl1 (l/min)	Crcl2 (l/min)	Crcl3 (l/min)	BP1	BP2	BP3	alb1 24hr/EMU	alb2 24hr/EMU	alb3 24hr/EMU	Dialysis days	TPE	Outcome Group
1	HUS	5	M	-/78/54	,	12	51		90/58	,		23/14	2/62	21	no	3
2	HUS	5	F	42/47/39	,	54			98/64	,	-/5	3/8			no	3
3	HUS	9	F	57/50/-	82	,	,	122/80	,		22/17				no	2
4	HUS	43	F	121/103/-	66	57	,	131/70	90/68		17/15	10/9		12	yes	3
5	HUS	63	F	159/112/105	24	50	50	138/80	120/70	130/80	20/18	16/13	3/3	NK	yes	3
6	HUS	65	F	136/93/88	46	58	76	148/96	160/80	180/100	24/21	22/13	39/131		yes	3
7	HUS	66	F	80/88/61	51	43	68	150/83	130/70	180/90	-/8	10/14	12/14		yes	3
8	HUS	73	F	111/124/105	111	45	34	172/68	160/70	180/80		8/4	8/9		yes	4
9	HUS	74	F	82/108/102	62	103	66	150/88	170/90	170/90		8/11	2/-		yes	3
10	HUS	74	F	111/93/92	33	30	51	138/88	120/70	130/70	4/3	5/5	11/6		yes	3
11	HUS	74	F	110/98/87	49	60	64	210/130	180/90	190/90	-/3	3/2	5/3		yes	3
12	HUS	77	M	109/114/100	9	30	24	206/110	190/90	204/100	54/66	27/16	22/13		yes	4
13	HUS	79	F	73/81/-	64	9		200/110	150/90		2120	543/434		12	yes	5
14	HUS	86	M	121/132/140	56	42	57	136/60	164/84	160/80	7/7	6/6	5/8		no	3
15	HUS	93	F	150/-/147	18		17	152/92		160/100	7/11		24/24		no	4
16	TMA	5	M	-/45/30		89	80		100/60	90/50	6/9	3/7	7/7		no	1
17	TMA	24	M	84/91/86	95	120		120/70	110/70		6/9	10/9			yes	1
18	TMA	63	F	92/78/71	41	68	97	136/80	120/70	120/74	6/12	14/-	8/5		no	1
19	TMA	77	F	136/140/151	31	34	35	236/128	180/90		25/28	14/18	18/-		no	4

Creatinine (cr), creatinine clearance (Crcl), blood presssure (BP), albumin (alb). 1,2,3 anniversaries. Shaded areas illustrate results outwith reference range

More than half of people aged over 60 years have a BP over 160/90mmHg, therefore blood pressure measurements in cases with HUS was also compared with controls. There were 32 BP measurements in cases with HUS and 127 BP measurements in controls aged more than 62 years. 17 (53%) of cases and 35(28%) controls had SBP over 159mmHg [(p=0.01) OR 3.0(1.3-6.6)]. 15 (47%) cases and 32 (25%) controls had DBP over 89mmHg, [p=0.029, OR 2.6 (1.2-5.8)].

On the third anniversary, 84 cases with uncomplicated gastrointestinal infection with *E. coli* O157 were matched for age and sex with one control subject. 61 of 84 were female and 23 male, the sex distribution reflecting the female preponderance in outbreak cases. The mean age of uncomplicated cases and matched controls was 58 years. The mean urinary Ccr in cases was 93 ml/min compared with 91 ml/min in controls (n.s.). 9(11%) cases and 7(9%) controls had a creatinine clearance < 80 ml/min/1.73m^2 (n.s.). 3(4%) cases and no controls had creatinine clearance < 25mls/min/1.73m^2. Five (6%) cases and five controls had increased urinary protein on 24-hr collection and EMU (>20mg/l). There were no differences in means or extremes of systolic or diastolic blood pressure between the two groups.

7.3.2 Outcome in children

Of 3 children who had HUS two had renal insufficiency and one minor abnormality at the end of the study period. One child with TMA had normal renal function. Of the five children attending the paediatric nephrologists, all were known to have normal renal function at the end of the study period.

13 children who had uncomplicated gastrointestinal infection participated in the study. One child had persistent microalbuminuria with albumin ranging from 26-62 mmol/l on five samples. Urinary tract infection was excluded and no reason for micralbuminuria was identified. Otherwise children who did not have HUS appeared to have normal renal function. There was a wide variation in Ccr measurements without supportive abnormality of renal function, which is likely to reflect difficulties in specimen collection in this age group.

7.4 Discussion

In the developed world *E. coli* O157 induced HUS is now established as the commonest cause of chronic renal failure in childhood. Over the past three decades, the spectrum of renal abnormality seen after HUS has been investigated in children. The results of published studies are difficult to compare and make inference from. These difficulties arise because the studies originate from different geographical areas with different epidemiological backgrounds, cover different time periods, report different proportions of diarrhoea associated HUS, extend to different periods of follow up and report different methods and definitions in the assessment of outcome of renal function (Table 7.1). Reduced GFR, increased urinary protein, significant microalbuminuria, hypertension, reduced renal functional reserve, chronic renal failure and ESRF have all been reported in varying proportions. The first reported experience of HUS was from Argentina [Gianantonio *et al.*, 1973]. 124 cases presenting between 1957-1972 were reviewed between five to thirteen years after presentation. 48% showed recovery, 18.5% had deterioration in renal function

of which almost half had progressed to ESRD, in the remainder there was minor abnormality. The proportion of these cases caused by *E. coli* O157 or other VTEC is a matter for speculation. Retrospective surveys of *E. coli* O157 isolates serotyped by public health laboratories in the UK, USA and Canada show that serotype O157:H7 was rare in diarrhoeal specimens during the 1970s. [Griffin and Tauxe, 1991]. More recent studies include a significant proportion of patients who have diarrhoea associated HUS (D+HUS). A recent study from the UK presented outcome of 114 patients with D+HUS [Small *et al.*, 1999]. 1 patient had ESRF, 5% had moderate CRF (Ccr 25-50 ml/min/1.73 m^2), 22 % mild CRF (Ccr 50-80 ml/min/1.73 m^2) and 72% normal renal function. Accumulation of all significant studies in the past three decades has shown mortality during the acute phase of 6% (range 2-17%). Of the patients who survive the acute illness, 73% (range 38-100%) recover normal renal function, 12% (1-31%) have minor abnormalities of renal function of uncertain significance at the time of reporting, 12% (2-28%) have significant abnormality of renal function and 3% progress to ESRF (1-16%), Table 7.1.

Follow-up data has largely focused on patients who have received dialysis. The development of chronic renal failure correlates with the number of days of dialysis and the duration of oligoanuria during the acute illness [Hughes *et al.*, 1991, Seigler *et al.*, 1991]. However there have been reports of progressive renal disease in patients who did not receive dialysis [Small *et al.*, 1999] had no apparent oligoanuria [Seigler *et al.*, 1991] and with apparently minor HUS [Gianantonio *et al.*, 1973].

The published rate of complication in children is the benchmark for expected outcome in adults. The numbers in our study are small but this is the only significant cohort of adults with *E. coli* O157 associated HUS. Of the 12 adults who survived HUS none had normal renal function, 1 had ESRD, one quarter had CRF the remainder and showed clear evidence of renal insufficiency. Comparison with the control group has shown significant differences between cases with HUS and controls in both Ccr and BP confirming that the measured abnormalities are not simply a reflection of the decline of renal function with age.

Eleven adults in this study were treated with TPE. It has been suggested in adults with HUS not related to *E. coli* O157 that TPE may limit the number of patients with chronic renal sequelae [Conlon *et al.*, 1995]. Our experience of TPE in adults with *E. coli* O157 associated HUS suggested that it influenced the course of HUS and improved prognosis during the acute illness. The small numbers involved make it impossible to assess the impact of this intervention in the late outcome of renal function in the cases presented.

Cases with TMA had no significant abnormality of renal function. Adult cases with uncomplicated gastrointestinal infection had the same prevalence of renal function abnormalities as the control population. There is therefore no suggestion that cases other than those who have had HUS require renal follow-up.

There has been uncertainty as to the optimal duration of follow-up of children with HUS. There is evidence that patients with normal GFR 2 years after HUS [De Jong

and Monnens, 1988] and normal protein/creatinine ratio in EMU at five years will with high probability keep normal renal function [Milford *et al.*, 1991]. Others however have shown a late deterioration of renal function after apparent recovery, and recommend follow-up examinations for at least 10 years [Tonshoff *et al.*, 1994, Siegler *et al.*, 1991]. The high prevalence of renal function abnormality in our patients suggests that all adults should be followed up after *E. coli* O157 associated HUS. The optimal duration of follow up remains uncertain. The risk of progression to ESRD is likely to be multifactorial and cumulative, influenced by the severity of the acute renal insult, age and comorbidity.

CHAPTER 8

Prevalence of the Irritable Bowel Syndrome and its Impact on Quality of Life to the Third Anniversary of Infection with *E. coli* O157

8.1 Introduction

Symptoms secondary to bacterial gastroenteritis were traditionally thought to be brief and self-limiting, but there is now growing evidence for the legacy of foodborne disease. Symptoms compatible with the irritable bowel syndrome (IBS) have been shown to occur after gastrointestinal infection with salmonella, campylobacter and shigella [McKendrick and Read, 1994, Neal *et al.*, 1997, Rodriguez and Ruigomez, 1999]. The largest study to date reported the prevalence of IBS to be 7%, six months after infection with campylobacter, salmonella or shigella [Neal *et al.*, 1997]. The only study to include a control group reported a prevalence of IBS of 4% at 1 year; the relative risk of IBS for cases was 10 times that of the control population [Rodriguez and Ruigomez, 1999].

Female sex [McKendrick and Read, 1994], the duration of diarrhoea during the acute illness [Neal *et al.*, 1997] and psychological factors [Gwee *et al.*, 1996] have been suggested as risk factors for the development of post infective IBS. Patients have been shown to have less psychiatric morbidity than those with idiopathic IBS and are thought to have a better overall prognosis [Chaudhary and Truelove, 1962, Harvey *et al.*, 1987]. IBS affects sleep, employment, sexual functioning, leisure, travel, diet and mood [Hahn *et al.*, 1999]. Recent publications have quantified the detrimental effect of idiopathic IBS on quality of life (QOL) [Gralnek *et al.*, 2000, Creed *et al.*, 2001].

The extent to which IBS occur after infection with *E. coli* O157 is not known. Acute gastrointestinal symptoms and complications of *E. coli* O157 are often severe. Haemorrhagic colitis occurs in 70% of cases and ischaemic colitis [Griffin *et al.*,

1990], appendicitis, oesophageal stricture and large bowel perforation are recognised complications of the acute illness [Morris *et al.*, 1991]. The severity of the acute illness suggests potential for chronic gastrointestinal problems and late colonic stricture formation has been described [Griffin *et al.*, 1990]. The study assessed the prevalence of gastrointestinal symptoms and IBS, annually to the third anniversary of infection, in patients who had *E. coli* O157 during the 1996 central Scotland outbreak. Gender and the severity of the acute illness were examined as risk factors for the development of IBS. Finally the impact of IBS on patients QOL was assessed.

8.2 Subjects and methods

8.2.1 Subjects

Subjects were patients infected with *E. coli* O157 in Lanarkshire during the 1996 central Scotland outbreak. Patients were invited for clinical review annually to the third anniversary of infection. At first review cases over 5 years of age were asked to complete a questionnaire with respect to their bowel habit before the *E. coli* O157 infection and their current bowel habit (Figure 8.1). The questionnaire was a self-completion questionnaire adapted from that validated by Neal *et al* [Neal *et al.*, 1997] in their assessment of IBS after bacterial gastroenteritis. Analysis of this data highlighted recall bias and reporting of bowel symptoms in children as possible sources of error. Therefore on the second and third anniversaries of infection analysis is confined to adult cases (15 years or older), for whom control subjects were sought. For each case ten possible age and sex matched control subjects, were generated from the CHI. Ultimately one matched control subject, with no history of

gastroenteritis, was recruited for each case. Control subjects completed the questionnaire on the second and third annual review.

8.2.2 Methods of diagnosis

A diagnosis of IBS was made from the questionnaire using modified ROME criteria [Thomson *et al.*, 1989] (Figure 8.2). In keeping with accepted criticisms of the ROME criteria [Camilleri, 1998] and the variable nature of IBS, subjects had difficulty specifying symptom frequency. A minimum symptom frequency was not therefore included within the definition of IBS. The history of cases acute symptoms was obtained from hospital notes, general practice records and by interview. In addition subjects completed a QOL questionnaire, SF-36 UK version, on the second and third anniversaries. The questionnaire was scored according to Medical Outcomes Trust guidelines [Ware, 1997].

One study reported pancreatitis in 23% of patients with *E. coli* O157 [Fukushima *et al.*, 1999]. Abdominal pain is integral to the definition of IBS and therefore it is possible that chronic pancreatic disease could have been mistaken for IBS. To exclude pancreatic disease, serum amylase and glucose was measured at review.

8.2.3 Statistics

Chi-squared test, Fishers exact test, Odds Ratios with 95% Confidence Intervals and the students t-test (2 tailed) are used for statistical comparisons between cases and controls.

8.3 Results

8.3.1 Demographic data

149 (67%) of the 224 Lanarkshire *E. coli* O157 cases, aged over 5 years, attended for clinical review on the first anniversary of infection and completed the bowel questionnaire. Cases included in the study were older than those who declined to take part, but there were no differences in severity of acute gastrointestinal symptoms between the those who participated and those who did not (Table 8.1).

8.3.2 Prevalence of IBS

On the first anniversary of infection the median age of cases was 58 years, 10 cases were aged between 5 and 15 years. 104 of 149 (70%) were female. The most frequently reported bowel symptoms were variation in bowel habit and abdominal pain, however all gastrointestinal symptoms were increased in prevalence compared to before infection. Before *E. coli* O157 infection 9 of 149 (14%) cases satisfied the ROME criteria for IBS. One year after infection 44 of 147 (29%) satisfied the modified ROME criteria, OR [3.4 (CI 1.8-55)], (Table 8.2). Within 1 year of infection 34 (23%) cases had developed new IBS, (Table 8.3).

On the second anniversary 103 of the 108 adult cases completing the questionnaire could be matched for age and sex with a control subject. The median age was 60 years. 38 of 103 (37%) cases and 11 of 103 (11%) controls satisfied the ROME criteria for IBS, [OR 4.89 (CI 2.3-10.3)], Table 8.2.

Figure 8.1: Bowel Questionnaire

Please complete questionnaire on your current bowel habit

	Yes	How many days a week	No
1. Do you suffer from abdominal pain?	☐ ⇒	☐	☐
2. Do you pass loose or watery stools (motions)?	☐ ⇒	☐	☐
3. Does your bowel habit vary from day to day?	☐ ⇒	☐	☐
4. Do you have to strain to open your bowels?	☐ ⇒	☐	☐
5. Do you rush to the toilet to open your bowels?	☐ ⇒	☐	☐
6. After opening your bowels do you ever feel the need to go to the toilet again?	☐ ⇒	☐	☐
7. Do you pass slime or mucous when opening your bowels?	☐ ⇒	☐	☐
8. Do you feel bloated or that your abdomen is swollen after eating a meal?	☐ ⇒	☐	☐
9. Do you have to loosen your clothing after eating a meal?	☐ ⇒	☐	☐

Figure 8.2: Modified ROME criteria for irritable bowel syndrome

Modified Rome criteria

Abdominal pain +

Irregular pattern of defecation (three or more of the following):

- Altered stool frequency
- Altered stool form (hard/loose)
- Altered stool passage (straining/urgency/sense of incomplete evacuation)
- Mucous per rectum
- Bloating or feeling of abdominal distension

On the third anniversary 93 of the 99 adults completing the questionnaire were matched with a control subject. All gastrointestinal symptoms compatible with the IBS were significantly more frequent in cases. 26 of 93 (28%) cases and 11 of 93 (12%) controls satisfied the modified ROME criteria, [(OR 3.0 (CI 1.3-6.3)], Table 8.2.

8.3.3 Risk factors for new IBS after *E. coli* O157 infection

To the third anniversary of infection 55 (37%) of cases had developed new IBS (Table 8.3). IBS appeared to occur more commonly in female patients, 43 of 104 (41%) females compared to 12 of 45 (27%) males developed IBS but this difference did not reach statistical significance. Age and indicators of severity during the acute illness (bloody diarrhoea, abdominal pain, >10 stools/day, diarrhoea > 7 days and vomiting) were not associated with development of new IBS within 3 years of infection.

8.3.4 Exclusion of chronic pancreatitis

On the second anniversary 9 of 103 (9%) cases and 13 of 103 (13%) controls had plasma glucose concentration of over 7.8 mmol/l. 6 (6%) of cases and 13 (13%) controls had serum amylase over 200 U/l. On the third anniversary 4 of 94 (4%) cases and 5 of 93 (5%) controls had an elevated plasma glucose. 8 of 94 cases (8.5%) and 11 of 93 controls (12%) had serum amylase over 100 U/l (new laboratory reference range). Therefore there were no differences in crude measurement of pancreatic exocrine or endocrine function.

Table 8.1: Demographic characteristics and clinical features of Lanarkshire *E. coli* O157 outbreak cases aged over 5 years

	Enrolled in study on first anniversary		
	Yes	**No**	**P-value**
Number of subjects	149	75	
Age (mean of years)	51	42	0.006
Stool culture positive	112 (75%)	59 (79%)	*
Female sex	104 (70%)	47 (63%)	*
Acute symptoms (outbreak case definition)			
Bloody diarrhoea	98 (66%)	48 (64%)	*
Non-bloody diarrhoea	33 (22%)	21 (28%)	*
Asymptomatic	18 (12%)	6 (8%)	*

*Not significant at a 5% level

Table 8.2: Gastrointestinal symptoms of *E. coli* O157 cases and control subjects.

	Cases Before O157 N (% of N)	Cases 1 year after O157 N (% of N)	OR (95% CI)#	Cases 2nd anniversary N (% of N)	Controls 2nd anniversary N (% of N)	OR (95% CI)•	Cases 3rd anniversary N (% of N)	Controls 3rd anniversary N (% of N)	OR (95%CI)•
Subjects	**149 (100%)**	**149 (100%)**		**103(100%)**	**103(100%)**		**93(100%)**	**93(100%)**	
Age (median years)		58	*	60	61	*	63	63	*
Female sex	104 (70%)	104 (70%)	*	72(70%)	72(70%)	*	68(73%)	68(73%)	*
Abdominal pain	23 (15%)	60 (40%)	3.7(2.1-6.7)	45(44%)	15(15%)	4.6(2.3-8.9)	37(40%)	17(18%)	3.0(1.5-5.8)
Loose/ watery motions	23 (15%)	50 (34%)	2.7(1.5-5.0)	44(43%)	17(17%)	3.8(2.0-7.3)	37(40%)	16(18%)	3.2(1.6-6.3)
Bowels vary day to day	38 (25%)	64 (45%)	2.2(1.3-3.7)	57(55%)	40(39%)	1.9(1.1-3.5)	52(56%)	29(30%)	2.8(1.5-5.1)
Strain	23 (15%)	37 (25%)	1.8(0.9-3.4)	38(37%)	19(18%)	2.8(1.4-4.9)	41(44%)	18(19%)	3.3(1.7-6.3)
Urgency	21(14%)	38 (26%)	1.8(1.1-2.9)	27(26%)	17(17%)	1.8(0.9-3.7)	34(37%)	22(24%)	1.9(1.0-3.5)
Incomplete evacuation	23 (15%)	43 (29%)	1.9(1.2-2.9)	40(39%)	16(16%)	3.5(1.8-8.8)	43(46%)	28(30%)	2.0(1.1-3.7)
Slime or mucous	19 (13%)	26 (18%)	1.4(0.8-2.4)	26(25%)	9(9%)	3.5(1.3-6.4)	26(28%)	11(12%)	2.9(1.3-6.3)
Bloated	28 (19%)	48 (32%)	1.7(1.1-2.6)	51(50%)	23(22%)	2.5(1.4-4.6)	49(53%)	29(31%)	2.5(1.4-4.5)
Loosen clothing	23 (15%)	30 (20%)	1.3(0.8-2.0)	41(40%)	16(16%)	2.6(1.3-5.0)	38(41%)	24(26%)	2.0(1.1-3.7)
IBS	**14 (9%)**	**44 (29%)**	**3.4(1.8-5.5)**	**38(37%)**	**11(11%)**	**4.8(2.3-10.3)**	**26(28%)**	**11(12%)**	**3.0(1.3-6.3)**

*not significant at a 5% level

 # statistical comparison of cases after and before infection

• statistical comparison of cases and controls

Table 8.3: Risk factors for the development of IBS after *E. coli* O157 infection

Risk Factor		Cases	New IBS Within 1 year of infection	OR (95%CI)	New IBS within 2 years of infection	OR (95%CI)	New IBS within 3 years of infection	OR (95%CI)
		N (%)	n1(%of N)		n2 (% of N)		n3 (% of N)	
Subjects		**149 (100%)**	**34 (23%)**		**48 (32%)**		**55 (37%)**	
Gender	F	104 (70%)	28 (27%)		38 (37%)		43 (41%)	
	M	45 (30%)	6 (13%)	*	10 (22%)	*	12 (27%)	*
Age	<=45	52 (35%)	11 (21%)		17 (33%)		21(40%)	
	>45	97 (65%)	23 (24%)	*	31 (31%)	*	34 (35%)	*
Bloody diarrhoea (mv=1)	Yes	103 (70%)	26 (25%)		35 (34%)		41(40%)	
	No	45 (30%)	8 (18%)	*	13 (29%)	*	14 (31%)	*
Diarrhoea (mv=1)	Yes	132 (89%)	31 (24%)		43 (33%)		49 (37%)	
	No	16 (11%)	3 (19%)	*	5 (31%)	*	6 (38%)	*
Abdominal pain (mv=7)	Yes	102 (72%)	25 (25%)		37 (36%)	*	42 (31%)	*
	No	40 (28%)	7 (22%)	*	9 (23%)		11(28%)	
Stool frequency > 10/ day	Yes	35 (32%)	7 (20%)		12 (34%)		14 (40%)	
	No	73 (68%)	21(29%)	*	28 (38%)	*	31(42%)	*
Diarrhoea duration > 7 days	Yes	38 (28%)	13 (34%)	2.4 (1.0-5.6)	17 (45%)		18 (47%)	
(mv=15)	No	96 (72%)	17 (18%)		25 (26%)	*	44 (46%)	*
Vomiting	Yes	40 (27%)	11 (28%)		15 (38%)		18 (45%)	
	No	109 (73%)	23 (21%)	*	33 (30%)	*	37 (34%)	*

* not significant at 5% level
mv= missing value

8.3.5 IBS and QOL

IBS in cases was clearly associated with reduced QOL. On the second anniversary cases with IBS had significantly lower mean scores in all eight SF-36 scales compared to cases without IBS; physical functioning ($p=0.01$), role physical ($p=0.024$), bodily pain ($p=0.01$), general health ($p=0.005$), vitality ($p<0.001$), social functioning ($p<0.001$), role emotional ($p<0.001$), mental health ($p<0.001$). Differences in SF-36 scores between the two groups were greatest in those scales which reflect mental health. On the third anniversary there was no significant difference on the physical functioning scale between cases with IBS and those without, however cases with IBS had persistently lower scores for the remaining scales; role physical ($p=0.015$), bodily pain ($p=0.001$), general health ($p=0.001$), vitality ($p<0.001$), social functioning ($p<0.02$), role emotional ($p<0.001$), mental health ($p<0.001$), Figure 8.3 a, b.

8.4 Discussion

This study shown that *E. coli* O157 infection is not only a cause of severe acute disease but is also responsible for significant ongoing gastrointestinal symptoms. One year after infection the relative risk of IBS is three times that prior to infection. The increased prevalence of IBS persists to the third anniversary of infection where the relative risk of IBS is three times that in the control group.

Figure 8.3a: Cases SF-36 scores on the 2rd anniversary

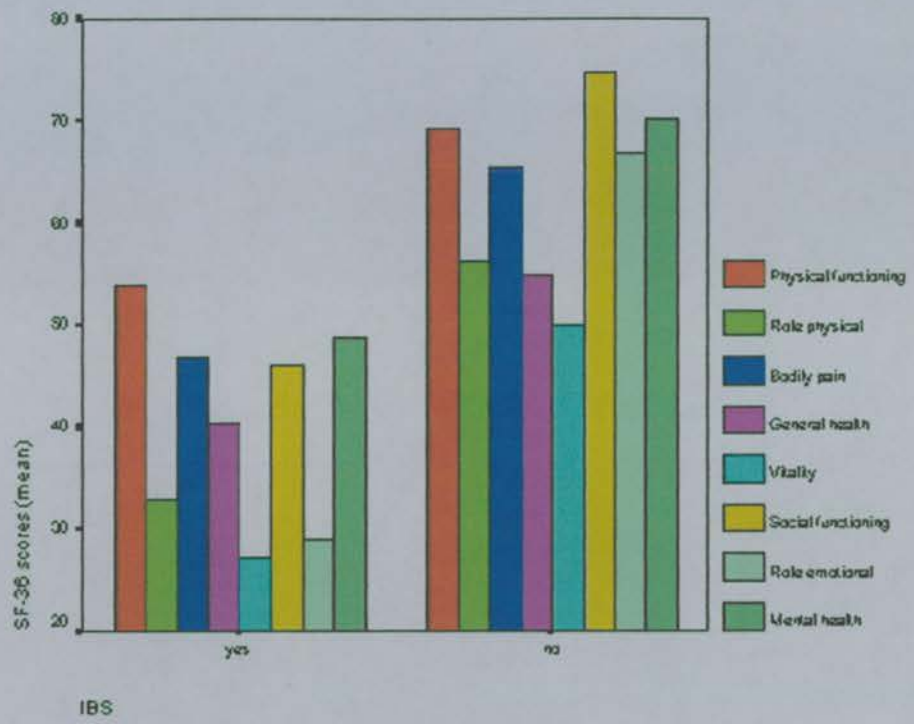
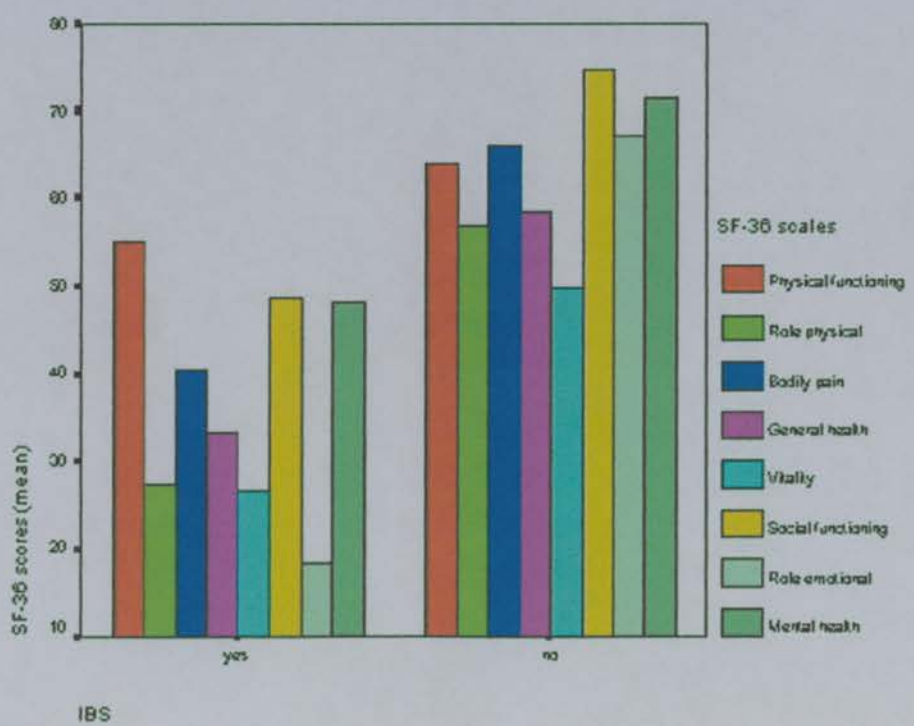


Figure 8.3b: Cases SF-36 scores on the 3rd anniversary



The risk of IBS after *E. coli* O157 seems to be higher than that after infection with other gastrointestinal pathogens. To the third anniversary of infection we documented new IBS in 37% of cases compared with the 4 to 7% rate of new IBS diagnosed after infection with campylobacter, salmonella and shigella [Neal *et al.* 1997, Rodriguez and Ruigomez, 1999]. The development of IBS does not appear to be related to the severity of gastrointestinal symptoms during acute infection. There was no evidence that chronic pancreatic disease contributed to patients symptoms. Four cases were referred by their general practitioners for barium enema or colonoscopy suggesting that their symptoms were sufficiently severe to warrant further investigation. No structural colonic pathology was identified.

The study may have over estimated the prevalence of IBS after *E. coli* O157 infection. There are four potential reasons for this, which have been addressed. Firstly female sex, an established risk factor for IBS, was over represented in cases infected with *E. coli* O157 during the central Scotland outbreak and consequently 70% of cases on the study were female. Secondly, because patients identified the variable nature of their symptoms, they were often unable to complete the part of the questionnaire that recorded symptom frequency, and we therefore did not require that patients specified a minimum symptom frequency to meet the modified ROME criteria for IBS. Thirdly, only two thirds of all outbreak cases of *E. coli* O157 agreed to participate in the study and we may have introduced a selection bias for subjects with ongoing symptoms. Finally, subjects who dropped out between the first and third anniversaries may represent cases with no symptoms. The methods addressed

each of these four areas of possible bias. The control population matched the case cohort in gender, thus excluding female sex as a source of bias. A separate (not presented) analysis of our data, that required a minimum symptom frequency of once per week, confirmed that on the second anniversary of infection all gastrointestinal symptoms were still significantly more prevalent in cases after *E. coli* O157 compared to controls and on the third anniversary abdominal pain and loose watery motions were significantly more common. Additionally the prevalence of IBS in the control population was within the range (8 to 21 %) expected for western adults [Jones and Lydeard, 1992, Thompson and Heaton, 1980] which confirms that the diagnostic criteria were adequate. With respect to bias introduced by attracting cases with ongoing symptoms, cases attended primarily for monitoring of renal function and the information sheet that accompanied the invitation for review did not focus on the possibility of ongoing gastrointestinal symptoms. Also cases who took part in the study were representative of all infected cases in terms of the severity of their acute gastrointestinal symptoms. Of the cases who were lost to follow–up, eight (25%) had IBS on the first anniversary review which suggests that the lost cases do not reflect the asymptomatic cases. Although the prevalence of IBS may still have been overestimated, female preponderance, the exclusion of minimum symptom frequency and "personal significance" bias could not explain the increase in prevalence of IBS after infection compared to before. This could have been explained by inaccurate patient recall of symptoms prior to infection, however patient recall of their symptoms prior to infection was not statistically different in the control group.

The development of IBS after *E. coli* O157 infection is likely to be multifactorial. In the first instance, a delayed return to normal of colonic function may occur after the initial destabilisation of the colonic microflora by the invading pathogen [King and Hunter, 1996]. Symptoms could ensue from altered bacterial fermentative activity with increased gas production. There are a number of theories on perpetuating factors. Studies have suggested that there is a subgroup of patients with IBS who have persistently increased concentrations of inflammatory cytokines including interleukin 1, which by inhibiting absorption of sodium and water could contribute to persistent diarrhoea [Collins, 1994]. Studies in animals have also shown that enteric infection can lead to persistent dysfunction of neuromuscular tissues [Collins *et al.*, 1992, Vallance *et al.*, 1994]. Bile acid malabsorption, which usually occurs in patient with disease of the terminal ileum or post cholecystectomy, has recently been shown to be associated with post infective IBS [Niaz *et al.*, 1997]. Gallbladder disease has now been documented in patients post *E. coli* O157 [Tarr, 1995] and bile acid malabsorption may be responsible for ongoing gastrointestinal symptoms in at least some of the patients after *E. coli* O157 infection. Psychological factors may also be important [Gwee *et al.*, 1996]. Patients who have IBS six months after infectious gastroenteritis have been shown to have higher scores for anxiety, depression, somatisation and neurotic trait than those who returned to normal bowel function.

A clear association between IBS post *E. coli* O157 and reduced QOL was demonstrated, with a measured detrimental effect on both physical and mental health. This is in keeping with recently published data on cases with idiopathic IBS, who have lower SF-36 scores than the U.S. general population in all scales, and lower

scores in physical scales than patients with diabetes mellitus and end stage renal disease [Grainek *et al.*, 2000]. The study findings offer insight into the legacy of gastroenteritis with *E. coli* O157, not only does it cause severe acute gastrointestinal symptoms, but also it generates chronic gastrointestinal symptoms which have a negative bearing on functional status and well-being.

There is conflicting evidence from small studies on the long-term prognosis of patients with an acute diarrhoeal onset of IBS. Two studies have shown that patients tend to have a good prognosis, either improving spontaneously or responding to a high fibre diet [Gwee *et al.*, 1996, Harvey, 1987]. However a third small study, which reviewed patients with IBS after salmonella to 5 years post infection, showed that 9 of 11 patients still had disturbed bowel function, and 7 out of 11 still had abdominal pain [McKendrick, 1996]. Our finding that the prevalence of IBS had further increased at two years and that a further 21 cases had developed IBS between the first and third anniversary suggests that IBS post *E. coli* O157 infection is unlikely to be a short term problem. It is therefore suggested that all individuals involved in this study should be followed up so that meaningful conclusions can be made about their long-term prognosis, both in terms of gastrointestinal dysfunction and their general physical and mental health.

CHAPTER 9

General Discussion

9.1 Introduction

Outbreaks are often fertile sources of new research questions and translating the questions into a research agenda is an important part of the outbreak legacy. Outbreaks in conception, however, are unpredictable and do not conform to traditional research design. The central Scotland outbreak was overwhelming for the patients affected, the responsible professionals and the local community; therefore some reservations on the data and the methods are inevitable. The conclusions drawn from the previous chapters have already been made. This chapter summarises the findings from the chapters with reference to the original objectives set out in the general introduction, explores the limitations of the study methods and the prospects for future research.

9.2 Risk factors for HUS in adults infected with *E. coli* O157

The identification of the population at risk of HUS determines their need for supervision and intervention and facilitates their access to appropriate health care settings. Children and older adults are known to be at greater risk of HUS and this was clearly demonstrated in the community and hospital studies of the central Scotland outbreak. The reasons for increased risk of HUS in these age groups remain unclear and subject to investigation. Initial speculation that it might reflect higher Gb3 concentrations at age extremes has not been verified [Boyd and Lingwood, 1989]. Our finding that cases on acid lowering drugs or with previous gastrectomy were at increased risk of HUS was unexpected. Hypochlorhydria may contribute to

the association of HUS with age as children and the elderly are known to have lower levels of gastric acid. The hospital study suggest that patients on acid lowering drugs should be closely observed irrespective of their age.

The association of preceding antibiotics with HUS requires verification in further studies. If the antibiotic was given for another infection in the days prior to acquisition of *E. coli* O157, subinhibitory concentrations may be found in plasma. Numerous studies have shown that subtherapeutic concentrations of quinolones, cotrimoxazole, cefixime, tetracycline and fosfomycin markedly increase the release of verotoxin [Waterspiel *et al.*, 1992, Karch *et al.*, 1986, Yoh *et al.*, 1999]. One study reported a 10-60 fold increase in VT production in the presence of subinhibitory concentrations of ciprofloxacin [Yoh *et al.*, 1999]. Subinhibitory concentrations of ciprofloxacin would be unusual in the context of active treatment as the recommended dose of quinolones are greater than 100 MIC, unless the VTEC has acquired resistance. This role of subinhibitory antibiotics *in vivo* is purely speculation, which the investigation of further outbreaks should clarify. In the meantime it seems reasonable to consider that those pre-treated with antibiotics are at increased risk of HUS.

The role of antibiotics in the treatment of *E. coli* O157 is equally complex. There is growing evidence that the effect of antibiotics may be dependant on their mechanism of action. This *in vitro* evidence has shown quinolones increase the release of verotoxin not only in subtherapeutic concentrations but also at concentrations used for treatment [Kimmitt *et al.*, 1999]. The hospital study was unable to further

implicate quinolones in the pathogenesis of HUS induced by *E. coli* O157. Current UK guidelines on the treatment of gastroenteritis recommend that elderly patients with bloody diarrhoea be treated with ciprofloxacin [Farthing *et al.*, 1996]. This might prove to be incorrect advice if the infecting organism is *E. coli* O157 and ciprofloxacin increases the risk of HUS in an already predisposed group. This clinical dilemma could be resolved by a randomised controlled clinical trial.

9.3 The earliest laboratory predictors for the development of HUS

Early identification of laboratory predictors of progression to HUS is vital and could select cases for trials of preventative and treatment therapies. A number of studies in children have shown that elevated WCC is associated with HUS. There has been only study, prior to those reported here, which made the observation that elevated WCC preceded HUS. The observation was made during an outbreak in children where 8 of 20 cases developed HUS [Pavia *et al.*, 1989]. Children who developed HUS had high WCC and fever on day three of illness, two days before the onset of recognisable HUS. The studies reported in chapters 3 and 4 clearly confirm that high WCC is a precursor to HUS, not only in children but also in adult cases.

The findings of an early rise in WCC will contribute to the investigation of the pathogenesis of HUS. The white cell response is reminiscent of leukemoid reaction seen in patients with HUS after *S. dysenteriae* type 1 [Koster *et al.*, 1978, Butler *et al.*, 1987]. The response would be consistent with toxin mediated activation of neutrophils and there is growing evidence that this is a central mechanism of HUS

[Forsyth *et al.*, 1989]. Neutrophilia at the onset of HUS correlates with poor prognosis; 50% of the variance of outcome in one study was explained by this parameter alone [Coad *et al.*, 1991]. At the 4th International symposium on VTEC, *in vitro* evidence was presented that VT2 binds exclusively to a single class of binding sites on neutrophils and it seems likely that this is the mode of transfer of VT from the intestine to endothelial cells [Monnens, 2000].

The studies presented in Chapters 3 and 4 also define more clearly the evolution of laboratory abnormalities. This work is the first to illustrate that falling platelets and haemoglobin, which are within the definition of HUS, are the last manifestations of the syndrome and cannot be relied upon for early detection.

During the Sakai City outbreak in Japan in 1996, retrospective analysis found an elevated C reactive protein (CRP) to be predictive of the development of HUS in children [Ikeda *et al.*, 1999]. In one study 46% of children with CRP greater than 2 mg/dl developed HUS [Kawamura *et al.*, 1999]. The measurement of CRP is now widely available in the hospital setting. CRP could prove additive to WCC in determining those at greatest risk of HUS. The value of CRP in determining prognosis should be investigated further.

The observation that hypoalbuminaemia is an early marker of HUS is new. It remains unclear whether this reflects severe gastrointestinal infection, systemic sepsis or is simply a manifestation of low albumin in older age groups. None of the

studies in children have reported albumin levels in patients with HUS. Future studies should evaluate albumin as this may prove an early indicator of incipient HUS.

9.4 Protocol for monitoring patients in the community to identify those at risk of HUS

Community monitoring in the central Scotland outbreak employed enormous resources already limited by the outbreak situation. It is impossible and unnecessary to monitor intensively all patients with disease symptoms. The studies reported in chapters 3 and 4 have identified risk factors for severe disease. A monitoring strategy can be proposed based on the expectation that complete HUS is usually established seven days from the onset of diarrhoea but progressive abnormalities are likely to be evident at an earlier stage.

For suspected *E. coli* O157 infection;

- All patients should have a stool culture performed.
- FBC, film, LDH, CRP, urea and creatinine should be checked at presentation if
  - aged <15 or >64 years.
  - aged 15-64 years with low gastric acid levels, immunosuppression or clinical evidence of severe disease.
- Patients with high WCC or red cell fragmentation should have all blood tests repeated every two days until 14 days after the onset of symptoms, unless all abnormalities clearly resolve during that time.

- Patients should be referred to secondary care if laboratory parameters indicative of HUS are clearly developing.
- Cases with normal blood results on presentation need have them repeated only if their clinical condition deteriorates.

This strategy is limited by observations from the studies;

- Patients aged between 15 and 65 years might develop HUS.
- 5 per cent of HUS patients never have an increased WCC response.

A monitoring protocol might not initially identify all patients who develop HUS; therefore, it is essential that all patients with suspected *E. coli* O157 infection are fully informed of potential complications and are cautioned to re-present for monitoring if their clinical condition deteriorates within the 14 days following the onset of their symptoms. This advice is limited by the fact that the early clinical features of HUS are non-specific and patients cannot be given clear guidelines based on symptoms alone.

The recommendations for monitoring patients with suspected *E. coli* O157 infection are based on current best evidence, but these could change with new information. Analysis of the Japanese outbreak found patients who developed HUS had high circulating levels of thrombomodulin and endothelin, compounds released by activated or damaged endothelial cells [Honda, 1999]. Although measurement of these compounds is currently a research technique, they could be incorporated into future clinical diagnostic procedures.

The main aim of employing a protocol in the management of future outbreaks of *E. coli* O157 infection is to identify as accurately as possible patients at high risk of developing HUS. It is important to specify the value of accurate prediction. Currently early diagnosis allows patients to receive early supportive therapy and potentially beneficial therapy such as TPE. Specific therapies based on knowledge of the pathogenesis of HUS are being evaluated [Armstrong *et al.*, 1995, Takeda *et al*, 1999].

9.5 The role of therapeutic plasma exchange in the treatment of adults who develop HUS in the context of *E. coli* O157 infection

The mortality associated with HUS in children has improved dramatically in the last two decades, and this has been attributed to improved dialysis techniques. It is unlikely that the mortality from HUS will be improved further by dialysis alone as most deaths are now due to neurological complications. Future improvement in outcome will be dependent on intervention that modifies the neurological complications of HUS.

The role of TPE in children with diarrhoea associated HUS has been controversial. A controlled trial of plasma infusion in children did not show benefit [Rizzoni *et al.*, 1986]. This was a study of 32 children with HUS, 17 of who received plasma and 15 symptomatic treatment only. There were no deaths in either group and the study had limited power to determine differences between the two treatment modes. TPE

involves removal of patients' plasma in addition to infusion of donor plasma. It is still unclear whether the benefit of TPE is due to removing an injurious factor from the patient's plasma, infusing a factor deficient in the patient but contained in the donor fresh frozen plasma (FFP) or both. In recent years TPE has been shown to be superior to plasma infusion in the treatment of idiopathic, drug induced and pregnancy associated TTP [Bell, 1997, Remuzzi and Ruggenenti, 1995, Rock *et al.*, 1991]. Therefore the accepted standard of care for idiopathic TTP is TPE, and this intervention has reduced mortality from 90% [Amorosi and Ultman, 1966] to between 10-20% [Bandarenko *et al.*, 1998]. There were no reports of the use of TPE in *E. coli* O157 infection.

There is concern that the pathogenesis of HUS differs, depending on the precipitant of the syndrome. There is concern that VTEC induced HUS is different in its pathology from the forms for which there is evidence that TPE is effective. It has been suggested that apoptosis and vWF multimers do not occur in VTEC induced HUS; therefore, TPE would not be effective [Laurence, 2000, Laurence and Mitra, 1997]. There is very little evidence on the pathogenesis of *E. coli* O157-induced HUS. Some investigators have demonstrated verotoxin induced apoptosis in human microvascular endothelial cells pre-exposed to TNF-α [Van de Kar and Monnens, 1998, Mahn *et al.*, 1996, Inward *et al.*, 1995], and large vWf multimeric forms have been shown to occur in plasma in *E. coli* O157-associated HUS [Moake *et al.*, 1994]. Perhaps more importantly, there is no consensus that the effectiveness of TPE is dependant on its ability to reverse apoptosis. The conflicting reports raise interesting questions about

the pathogenesis of HUS, but they present no definitive evidence that aetiological distinction is related to pathological distinction and, therefore, response to TPE.

To date TPE has not been subjected to clinical trials in *E. coli* O157-related HUS. In adults mortality from VTEC associated HUS was recorded at 88%, and commensurate with early outcomes from idiopathic forms of HUS. In adults in the Lanarkshire outbreak, TPE was a valuable intervention; however, this was not a randomised, controlled clinical trial. During the 1996 *E. coli* O157 outbreak in Japan, 4 of 12 children who developed HUS in one hospital received only plasma exchange. All 4 had a favourable outcome and 3 of the 4 improved promptly after the introduction of TPE [Fukushima *et al.*, 1999]. VTEC associated HUS in children now generally responds to dialysis and TPE might not be routinely indicated in this age group. There is growing evidence for the effectiveness of TPE in all age groups, and there is a stronger case for use of TPE in adults among whom mortality remains high.

The clinical efficacy, or otherwise, of TPE in *E. coli* O157-associated HUS needs to be determined definitively. Certainly there is no convincing evidence, from current understanding of the pathogenesis or clinical evidence, that TPE could not be effective. Enhanced surveillance of HUS in Scotland will soon be implemented by SCIEH. Similar surveillance was previously operative in children throughout the UK. HUS surveillance in Scotland will be a clinically driven system and it is hoped that it will provide prognostic clinical and treatment outcomes, which will assist cost benefit analysis of intervention strategies, particularly the role of TPE.

9.6 Genetically mediated inflammatory responses of individuals associated with the severe disease

In Chapter 6 two genetic markers were identified in significantly higher proportions of patients in the Lanarkshire outbreak, blood group O and absence or weak expression of the P1 antigen or erythrocytes. The association with group O does not appear to be related to increased bacterial binding to epithelial cells as has been observed for *H. pylori*. Over 80% of patients with HUS had no or low levels of P1 on their erythrocytes. *In vitro* TNFα responses induced by culture filtrates of the outbreak strain from leukocytes of blood donors whose erythrocytes were P1-negative were significantly higher than those of P-positive donors.

Approximately 75-80% of the normal Caucasian population express blood type P1 on erythrocytes but the antigenic strength varies considerably. The P antigen is structurally similar to Gb3 the verotoxin receptor. It is expressed only on a few cell types, particularly on erthrocytes, monocytes, some parts of the vascular endothelium, and on renal glomerular cells of infants. The sites and strength of expression vary with age. The association with weak or absent expression of P1 and HUS would be expected from our understanding of verotoxin receptors. Contrary to our findings, a study from Japan found no association between P1 blood group antigen and HUS; however, the prevalence of P1 blood group in Japan is low (35%) and the study was small, factors which may have limited the conclusion [Ashida *et al.*, 1999].

There is compelling evidence for cytotoxic and inflammatory events in the pathogenesis of HUS, and it is reasonable to look for links between them. Both lipopolysaccharide and the cytokines TNFα and interleukin 1β stimulate human glomerular endothelial cells to increase the surface expression of verocytotoxin receptors thus making them more vulnerable to cytocidal effects [van Setten *et al.*, 1997]. Just as inflammatory events have the potential to increase cytotoxicity, so too is there a pathway by which verocytotoxin can induce an inflammatory event and several studies have found high levels of TNFα, IL-6 and IL-8 in the plasma and urine of children with HUS. High levels of TNFα (> 100 IU/ ml) produced by leukocytes of P1 negative donors suggests that P1 blood group is indeed important in VTEC induced HUS. This observation and the significant excess of group O among the patients who died need to be examined in future studies.

9.7 Chronic renal function abnormalities in adults after *E. coli* O157 induced thrombotic microangiopathy

Chronic renal disease occurred after HUS in the population studied [Chapter 7]. The implications for the cases presented and overall prognosis cannot yet be determined. No case was dependant on dialysis at the time of reporting. With longer follow up the abnormalities detected may become clinically important. It is reasonable to speculate that if the histological abnormalities detected in children are present in adults, renal decline is inevitable and this decline could be exaggerated by atherosclerosis in older age groups. Annual review of renal function for all patients

with HUS is recommended. Enhanced surveillance of HUS in Scotland may give further insight into the long term prognosis of VTEC induced disease in adults.

9.8 IBS after *E. coli* O157

This is the first study to identify IBS after *E coli* O157 [Chapter 8]. The severity of acute disease and reports from other pathogens suggested that chronic gastrointestinal symptoms could occur. A large proportion of cases developed IBS after disease due to *E coli* O157. The study could have over estimated the prevalence of this complication, but IBS appears to be more common after *E. coli* O157 infection than after disease due to other gastrointestinal pathogens.

Ongoing gastrointestinal symptoms may be functional, a legacy of colonic inflammation, perpetuated by bile acid malabsorption or pancreatic exocrine insufficiency. The study assessed bowel symptoms up to three years after infection, and in that time no structural colonic lesion was identified in the small number of patients who underwent further investigation. Colonoscopic examination during acute *E. coli* O157 infection shows right sided disease. The histological features are a mixture of ischaemic and infectious changes [Griffin *et al.*, 1990]. Ischaemic colitis is a recognised precursor to colonic stricture formation [Simi *et al.*, 1995] and colonic strictures have been described following disease due to *E. coli* O157. One small study of diarrhoea and bile acid malabsorption confirmed gastrointestinal infection with campylobacter, salmonella or shigella in six of 84 cases [Niaz *et al.*, 1997]. Bile acid malabsorption may play a greater role in diarrhoea after E. coli

O157 as gallbladder disease has been confirmed during the acute illness. Therefore in some of our patients, it is possible that their symptoms are not purely functional but a precursor for later disease and a low threshold should exist for further investigation of ongoing gastrointestinal symptoms.

9.9 The effect of *E. coli* O157 infection on future quality of life

A reduced quality of life was noted among the patient population studied, and this can be linked to symptoms of IBS. IBS is not life threatening but has a significant impact on medical resources. In our patients, all SF-36 scales were lower than those reported in subjects in the UK with idiopathic IBS [Akehurst *et al.*, 2002] (figure 9.1). The reasons for this observation remain unclear, perhaps our patients have lower QOL because their gastrointestinal symptoms are not purely functional. All SF-36 scales were lower in our patients without IBS than UK normative data, an observation that might be explained in part by the fact that this group included 6 cases who had HUS.

9.10 Costs

E. coli O157 imposes considerable cost on the physical and mental well being of the affected individuals. The financial costs are also substantial and cannot be ignored. Study of an outbreak from Scotland in 1994 investigated cost of complications [Roberts *et al.*, 2000]. The average cost per patient who developed HUS was £62 353 compared with £1030 in those who had uncomplicated disease. The cost per

hospitalised patient was £8417. The cost of TPE was estimated as £2500 per patient. It is impossible to put a price on the cost of premature death. The cost of chronic renal disease and IBS cannot yet be predicted as the severity and prevalence of these complications is presently unclear. When the consequences of infection are so enormous all efforts should be targeted at prevention. There is evidence that preventative strategies implemented after the central Scotland outbreak are taking effect.

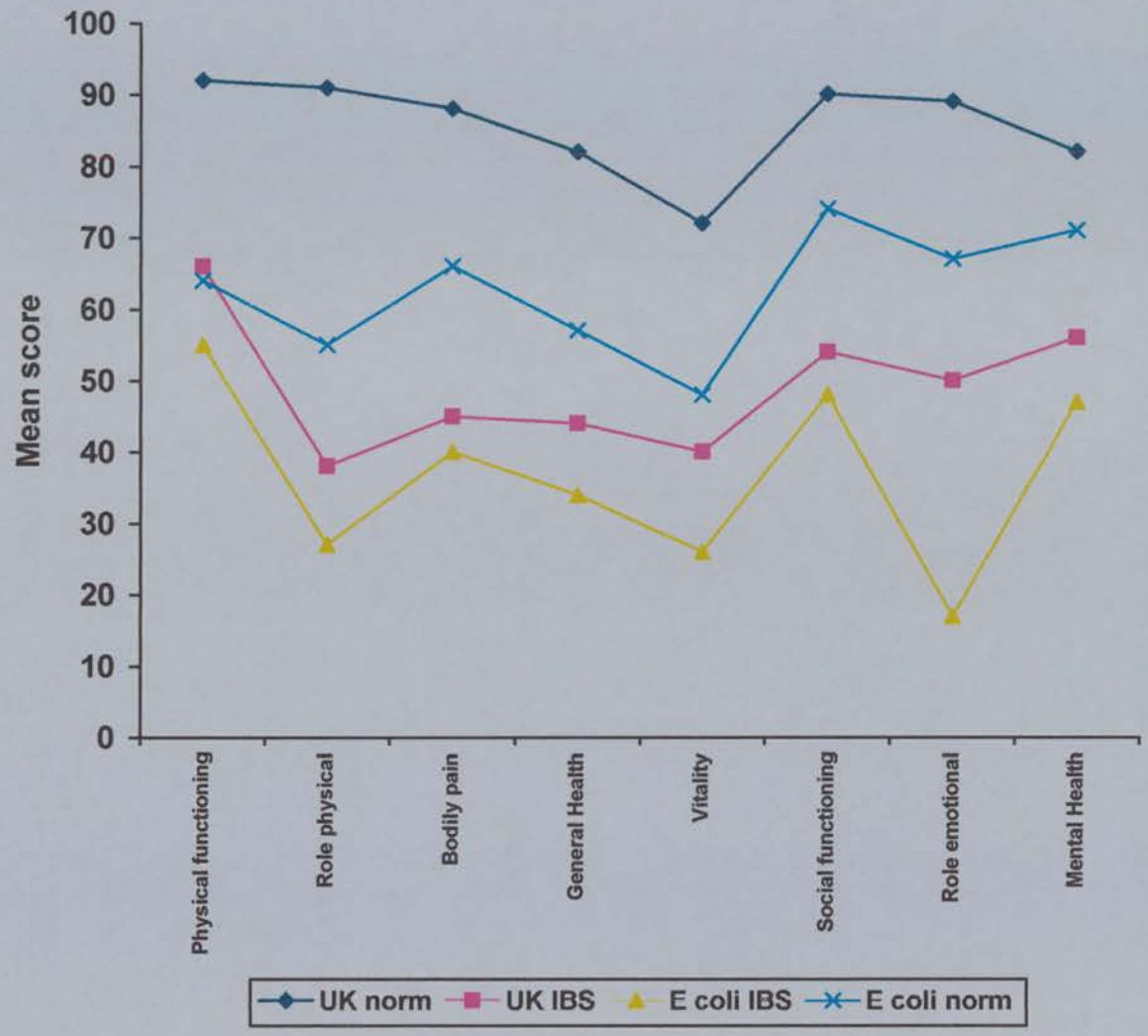
9.11 Future studies

The information presented identifies areas for future research:

1. Effects of subinhibitory levels of different classes of antibiotics on release of endotoxin as well as VT and the effects of these on inflammatory responses.
2. Effect of low levels of gastric acid on release of VT.
3. Correlation between high CRP and WCC and inflammatory responses (IL-8, TNF, IL-1B, IL-10) in serum of patients.
4. Combined predictive value of CRP and WCC in determining risk of HUS.
5. Albumin levels in children as a marker of severe disease or HUS.
6. Prospective study of all adults with HUS to determine acute and chronic outcomes and effectiveness of TPE.
7. Randomised controlled clinical trial of ciprofloxacin in adults with *E. coli* O157 to determine risk of HUS.
8. Interactions between different endotoxin O types and VT in relation to induction of pro-inflammatory cytokines.

9. Assessment of pro- and anti-inflammatory gene polymorphisms among patients with *E. coli* O157 disease, and relation of severity of disease to inflammatory mediators in serum during acute stage of the infection.
10. Measurement of IL 1 in patients with ongoing gastrointestinal symptoms.
11. Measurement of faecal bile acids and faecal pancreatic enzymes in patients with ongoing gastrointestinal symptoms.

Figure 9.1 SF-36 scales in idiopathic and *E. coli* O157 related IBS and normative data



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Monitoring patients in the community with suspected *Escherichia coli* O157 infection during a large outbreak in Scotland in 1996

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SUMMARY

During outbreaks of *Escherichia coli* O157 a minority of patients with suspected infection develop haemolytic uraemic syndrome (HUS). The ability to identify this subgroup at an early stage is beneficial as mortality from HUS is high and may be influenced by intervention. During the 1996 Central Scotland *E. coli* O157 outbreak, of 886 patients from the community with suspected infection monitored at an outbreak clinic, nine developed HUS. We assessed factors associated with the development of HUS in this group. Children and the elderly were at increased risk of HUS. However, high white cell count was as least as good a predictor of HUS as age. High white cell counts predicted development of HUS with a sensitivity of 89%, specificity of 87%, positive predictive value of 7% and a negative predictive value of over 99%. We have used the results from this study along with other currently available evidence to propose a monitoring protocol for patients from the community with suspected *E. coli* O157 infection.

INTRODUCTION

Since *Escherichia coli* O157 (*E. coli* O157) was first recognized as a cause of gastroenteritis in 1982 [1] it has become increasingly identified as a significant threat to the public health. Of patients with symptomatic infection approximately half have non-bloody diarrhoea whereas the remainder develop haemorrhagic colitis [2]. An estimated 2–7% of patients with symptomatic infection develop the life-threatening renal complication, haemolytic uraemic syndrome (HUS), or neurological complications, previously referred to as thrombotic thrombocytopenic purpura (TTP) [3]. HUS and TTP are different clinical manifestations of the same pathological microvascular process, due to systemic absorption of the Shiga toxin produced by *E. coli* O157 [4, 5], and are now uniformly referred to as HUS in the context of this infection.

Scotland has one of the highest incidences of reported *E. coli* O157 infection in the world [6]. Whilst most reported cases are sporadic [7] outbreaks continue to occur. In 1996 the largest outbreak to date in the United Kingdom occurred in central Scotland [8]. The outbreak involved 512 cases (337 confirmed or probable cases), of whom 34 developed HUS and 22 died (17 deaths were considered to be directly attributable to *E. coli* infection at the Fatal Accident Enquiry that followed the outbreak).

Early in the course of the outbreak the source of infection was traced to a butcher's shop in Wishaw, a town in Central Scotland with a population of approximately 50000. The majority of cases lived in Wishaw and the surrounding area and understandably there was a great deal of public anxiety about the

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Table 1. *Case definition status of the 245 cases monitored at the community clinic*

Symptoms	Stool specimen positive*	Stool specimen negative†	
		Serology positive‡	Serology negative†
Asymptomatic or no history	Confirmed (19)	Possible (23)	Not a case
Non-bloody diarrhoea only	Confirmed (51)	Possible (15)	Not a case
Bloody diarrhoea and/or HUS	Confirmed (72)	Probable (28)	Possible (37)

* For the outbreak strain (*E. coli* O157:H7, phage type 2, verocytotoxin type 2 producing, DNA profile by pulse-field gel electrophoresis characteristic of the outbreak strain) either by primary culture or immunomagnetic separation.
† Or not done.
‡ Or post-mortem evidence of infection with the outbreak strain.

outbreak within the town. To ease pressure placed on local primary- and secondary-care services a special community outbreak clinic was established in the local health centre. The clinic aimed to support the public health investigation, facilitate the provision of consistent public health advice to minimize secondary spread of the infection, and co-ordinate the care of cases.

The outbreak clinic was principally used to monitor patients from the local community with clinically suspected *E. coli* O157 infection. A patient with onset of diarrhoeal illness since the start of the outbreak was defined as having suspected infection and these patients could be referred to the clinic by their GPs. In addition the clinic was also used by patients required to submit samples for work exclusion purposes, and to follow up after discharge a number of known cases who were admitted to hospital early in the course of the outbreak.

A protocol to guide the management of patients with suspected *E. coli* O157 infection was drawn up at the start of the outbreak and was used consistently at the clinic. The protocol specifically aimed to confirm that patients fulfilled the case definition, ensure early diagnosis of HUS, and facilitate prompt and appropriate referral of patients requiring secondary care.

Stool microbiology and paired serology were performed to establish whether patients fulfilled the case definition (Table 1). Stool culture on Sorbital McConkey agar was performed locally, but other tests such as stool Immunomagnetic Separation, and serology were carried out at the Scottish *E. coli* O157 reference laboratory in Aberdeen. Due to delays in receiving final results from the reference laboratory, at the time patients were attending the clinic, whether they fulfilled the case definition was often still unknown.

To monitor for HUS, all patients with suspected infection had their haemoglobin, white cell count (WCC), platelets, blood film, lactate dehydrogenase level (LDH), and serum urea and creatinine checked every 2 days for 14 days. Standard criteria were developed to guide referral to secondary care. Referral was recommended for patients with severe clinical manifestations of infection (such as dehydration requiring parenteral fluid management) and patients whose laboratory findings indicated the imminent development of HUS (such as a rising LDH level of falling platelets) [8].

Previous studies have identified risk factors for HUS in patients with known *E. coli* O157 infection. Young children and the elderly have consistently been found to be more likely to develop HUS than young adults [2, 3, 9]. Studies involving children have suggested that a raised WCC early in the course of illness is also associated with an increased risk of HUS [10–12]. A high WCC has also been consistently shown to be an adverse prognostic indicator in hospitalized children with HUS secondary to *E. coli* O157 infection [13–15]. WCC in adults with *E. coli* O157 has never been assessed.

Gender, the presence of haemorrhagic colitis or fever, or the use of antimotility agents have been inconsistently associated with higher risk of HUS [2, 3, 11]. Retrospective analysis of all hospitalized cases from the Central Scotland outbreak suggested that patients taking antacids were at increased risk of HUS [16]. There is a lack of agreement regarding the role of antibiotics in HUS [17, 18] and it now seems that antibiotics of specific classes will have harmful and beneficial effects.

All the data relating to the patients monitored at the Wishaw clinic during the central Scotland outbreak have been validated and linked by the Information and Statistics Division of the NHS in Scotland [19]. The data include patients' demographic features, limited clinical information such as the presence or absence of bloody diarrhoea, and all microbiological, biochemical, and haematological results. The data provide a unique opportunity to examine the features associated with the subsequent development of HUS in patients from a wide age range with suspected *E. coli* O157 infection.

We examined the ability of age and patients' WCC to predict the development of HUS in suspected infection. We have not examined other clinical features in detail. A separate analysis of all cases hospitalized during the central Scotland outbreak has shown no other significant association between these clinical features and subsequent development of HUS [16].

SUBJECTS AND METHODS

Patients with suspected *E. coli* O157 infection (but not with established HUS) who were referred from primary care to the Wishaw clinic for monitoring were included in the study.

The case definitions employed during the outbreak and the number of cases meeting each definition are shown in Table 1. For this study a case was defined as any patient meeting the confirmed, probable, or possible case definitions.

During the outbreak and for the purposes of this study HUS was defined as follows:

(i) red cell fragmentation on blood film, and lactate dehydrogenase > 1·5 times the upper limit of normal;
(ii) thromocytopenia (platelets $< 150 \times 10^9/1$);
(iii) acute renal impairment (urea and creatinine above the normal range and rising) and/or new neurological signs.

The day on which all three criteria were met was deemed the day of onset of HUS.

The socio-economic status of patients was assessed by converting postcodes of residence into deprivation categories using the Carstairs and Morris index [20]. Deprivation categories are ranked 1–7, with 7 representing the most deprivation.

We first assessed the association in patients with suspected infection between age, gender, and presence of bloody diarrhoea, and subsequent development of HUS. We next mapped the clinical course of the patients who developed HUS in detail to identity which laboratory parameters became abnormal first. We then specifically assessed how well the WCC predicted the development of HUS in patients with suspected *E. coli* O157 infection.

The sensitivity, specificity, and predictive values for a patient having one or more abnormally high WCC prior to the onset of HUS were calculated. An abnormally high result was defined as one above the age appropriate reference range specified by the local laboratory (reference ranges available on request). The exact method for calculating a confidence interval for a single sample proportion was used to calculate 95% confidence intervals for all results [21]. The analysis was repeated using neutrophil count rather than total WCC. Finally we assessed how well age and WCC considered together identified patients at highest risk of developing HUS.

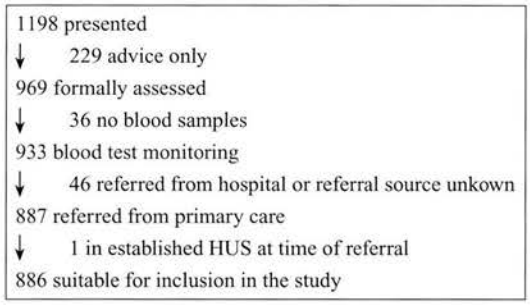

Fig. 1. Community clinic patients eligible for inclusion in the study.

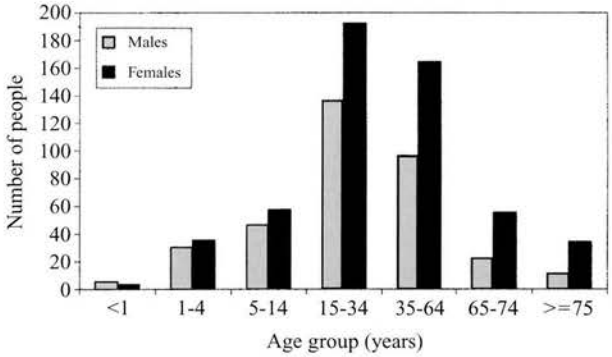

Fig. 2. The age and gender distribution of patients monitored at the community clinic.

RESULTS

In total 1198 people presented to the Wishaw clinic. Of these 229 did not meet the clinic's criteria for

Table 2. *The proportion of patients (cases) monitored at the community clinic that developed HUS; by presence of bloody diarrhoea*

Bloody diarrhoea	Number of patients (cases) monitored at the clinic	Number that developed HUS
Present	133 (133)	6 (6)
Absent	753 (112)	3 (3)
Total	886 (245)	9 (9)

Fisher's exact test for all patients, $P < 0{\cdot}001$.
Fisher's exact test for cases, $P = 0{\cdot}514$.

assessment. A further 36 people had no blood samples taken. This group includes people required to submit microbiological samples only for possible work exclusion purposes. Therefore, 933 people underwent blood test monitoring according to the clinic protocol. Of the 933 patients 39 were referred from hospital for post discharge follow up. For seven patients it was unknown whether they were referred from primary or secondary care. The remainder were known to be referred from primary care. One person referred from primary care had established HUS at the time of referral so no information could be obtained on the features predating the development of HUS. Overall 886 patients were eligible for inclusion (Fig. 1).

This group is representative of patients presenting to primary care with suspected *E. coli* O157 infection during a community based outbreak. Of the 886 patients monitored, 245 were cases, of which 170 (69%) had confirmed or probable infection (Table 1). Twenty-seven of the cases were admitted to hospital, 9 developed HUS, and 2 died.

The age and sex distribution is shown in Figure 2. Of the patients monitored 89% were resident in areas assigned deprivation category 5 or 6. This reflects the fact that Wishaw and its surroundings is a relatively deprived area of Scotland.

Men (3/346) and women (6/540) monitored at the clinic were equally likely to develop HUS (Fisher's exact test $P = 1{\cdot}0$). Children < 15 years and adults > 64 years (7/298) however were significantly more likely than adults aged 15–64 years (2/588) to develop HUS (Fisher's exact test $P = 0{\cdot}008$).

The presence of bloody diarrhoea was significantly associated with development of HUS in all clinic attendees. However, bloody diarrhoea was not found to be significantly associated in the subset of persons fulfilling the case definition (Table 2). Three of the nine cases that developed complications progressed straight from non-bloody diarrhoea to HUS without having haemorrhagic colitis.

In terms of assessing the ability of laboratory parameters to predict HUS, we initially examined in detail the clinical course of the nine patients that developed complications (Table 3). The median interval between onset of symptoms and onset of HUS was 9 days (range 5–15). In general an elevated WCC preceded the development of HUS and also preceded changes in urea, creatinine, LDH, haemoglobin, and platelet levels, and the appearance of fragmented red cells (Table 4).

Eight of the nine patients with HUS had a high WCC at some point during their illness. In all eight the WCC became abnormal before the onset of HUS, a median of 1·5 days after the onset of symptoms, and 5 days (range 1–8) before the onset of HUS. In 7 of the 8 patients (the exception was a patient who did not develop a raised WCC until 14 days after the onset of symptoms) the WCC was abnormal on the first blood sample, obtained when the patients presented. These findings are compatible with results from the hospitalized cases, in whom the WCC on day two of illness was significantly higher in cases who developed HUS, preceding changes in other laboratory markers by several days [16].

The presence of one or more high WCC results predicted the subsequent development of HUS in all clinic attendees, with a sensitivity of 89%, specificity of 87%, positive predictive value of 7%, and negative predictive value of over 99% (Table 5). A high WCC similarly predicted the subsequent development of HUS in the cases, monitored at the clinic (Table 5).

As it is specifically neutrophils that are implicated in the pathophysiology of complicated *E. coli* O157 infection [22, 23], we also assessed how well the neutrophil count predicted the development of HUS. We found that the neutrophil count was not significantly better than the total WCC in predicting HUS.

Finally, as age group and WCC are the features most strongly associated with the subsequent development of HUS, we assessed the predictive value of

Table 3. *Demographic and clinical details of the nine cases that developed HUS**

Case no.	Age	Sex	Blood in stool	Case definition	Clinic	HUS	WCC	Hb	Fragmented red cells	LDH	Platelets	Urea	Creatinine
1	70	F	Yes	Confirmed	0	5	0	5	3	4	5	2	5
2	70	M	Yes	Confirmed	1	5	1	5	5	1	5	1	N
3	63	F	Yes	Confirmed	3	6	1	1	3	3	6	3	N
4	10	M	No	Confirmed	2	7	2	N	7	4	7	7	2
5	6	F	Yes	Confirmed	1	9	1	N	7	1	9	7	1
6	80	F	No	Confirmed	4	11	4	11	8	8	11	8	N
7	78	F	Yes	Probable	5	11	5	8	8	5	10	5	5
8	61	F	Yes	Confirmed	6	12	N	12	12	10	6	10	10
9	2	M	No	Confirmed	2	15	14	6	15	10	15	15	2

* Clinic, day of first attendance at clinic; HUS, day of onset HUS: All blood results, day of first recorded abnormal result (high or low as appropriate); Note all results are based on the day of onset of symptoms being day 0. N, indicates that no abnormal result for that blood parameter was recorded for that patient at any point during their illness (either before or after the onset of HUS).

Table 4. *Median interval between onset of symptoms and first abnormal result in cases with HUS*

	Number of patients with an abnormal result recorded at some point during the course of their illness	Median interval between onset of symptoms and first recorded abnormal result (days)	Range (days)
WCC	8/9	1·5	0–14
Haemoglobin	8/9	7	1–12*
Fragmented red cells	9/9	7	3–15
LDH	9/9	4	1–10
Platelets	9/9	7	5–15
Urea	9/9	7	1–15
Creatinine	6/9	2	1–10

Day 0 is day of onset of symptoms.
* Note that 1 of the 8 patients who developed anaemia only did so after the onset of their HUS.

Table 5. *The validity of a high white count result in predicting subsequent development of HUS in all patients (cases) monitored at the community clinic*

High white count	HUS	No HUS	Total
Present	8 (8)	113 (49)	121 (57)
Absent	1 (1)	764 (187)	765 (188)
Total	9 (9)	877 (236)	886 (245)
Summary of results for all patients			
Sensitivity:	88·9% (51·8–99·7%)		
Specificity:	87·1% (84·9–89·3%)		
Positive predictive value:	6·6% (2·9–12·6%)		
Negative predictive value:	99·9% (99·3–100%)		
Summary of results for cases			
Sensitivity:	88·9% (51·8–99·7%)		
Specificity:	79·2% (74·1–84·4%)		
Positive predictive value:	14·0% (6·3–25·8%)		
Negative predictive value:	99·5% (97·1–100%)		

age group and WCC combined. The positive predictive value of age (< 15 or > 64) alone was 7/298 (2·3%, 95% CI 0·9–4·8%); that of WCC alone was 8/121 (6·6%, 2·9–12·6%); and that of age and WCC combined was 7/50 (14%, 5·8–26·7%).

DISCUSSION

This study is unique in assessing features associated with the development of HUS in a large number of patients, from a wide age range, with suspected *E. coli* O157 infection monitored in a community setting during a large-scale outbreak.

We found that children and the elderly with suspected infection are at higher risk of developing HUS than young adults. This agrees with previous work involving patients with known *E. coli* O157 infection [2, 3, 9]. We found that bloody diarrhoea is not a good predictor of HUS and this too is in agreement with previously published work [24].

Significantly, this study also demonstrates the importance of a raised WCC as a predictor of HUS, in patients of all ages with suspected as well as confirmed *E. coli* O157 infection. Raised WCC is possibly a better predictor of HUS than age. Patients with normal WCC are at very low risk of HUS.

Additionally development of a high WCC precedes changes in other laboratory parameters in HUS. This finding is in keeping with the fact that neutrophils carry Shiga toxin and therefore are likely to be pivotal to the pathogenesis of HUS [23]. Our findings thus confirm the value of laboratory monitoring in patients with suspected *E. coli* O157.

The clinic was invaluable in alleviating pressure on local services, and ensuring that patients received consistent information and were monitored and referred on to secondary care in a consistent way. In addition it facilitated comprehensive data collection. We would recommend the establishment of a similar clinic during any large community based *E. coli* O157 outbreak.

The protocol used at the clinic required very comprehensive monitoring of all patients with suspected infection, including patients that we could now identify as being at very low risk of developing HUS. We would therefore, in future, recommend the following streamlined monitoring protocol for patients in the community with suspected *E. coli* O157 infection, targeting those at extremes of age who present with high WCC:

(i) All patients should have a stool culture performed.

(ii) All patients aged < 15 or > 64 years, and adults aged 15–64 years with low gastric acid levels, or who clinically appear systemically unwell, should have their full blood count and film, LDH, and serum urea and creatinine checked at presentation. This will identify patients with a raised WCC (indicating increased risk of HUS) and those with established HUS.

(iii) Patients with a raised WCC or other significant abnormality such as evidence of haemolysis should be clinically reviewed and have all blood tests repeated every 2 days until 14 days after the onset of symptoms, unless all abnormalities clearly resolve during that time.

(iv) Patients should be referred to secondary case if laboratory parameters indicative of HUS are clearly deteriorating.

Neither this nor any other monitoring protocol will identify all patients who develop HUS. Hence it is essential that in addition all patients with suspected *E. coli* O157 infection are fully informed of potential complications and are cautioned to represent for monitoring if their clinical condition deteriorates within the 14 days following the onset of their symptoms, since the early clinical features of HUS are non-specific.

Whilst we believe that these recommendations are based on current best evidence we recognize that the situation may develop in the future. For example, during an extremely large *E. coli* O157 outbreak in Japan in 1996, retrospective analysis found an elevated C reactive protein (CRP) to be predictive of the development of HUS in children [25]. CRP may therefore prove to be a useful additional monitoring tool for the management of future outbreaks, and its potential role should be evaluated further. Unfortunately, however, laboratory methods for the measurement of CRP are not currently standardized throughout the United Kingdom.

Another analysis of the same Japanese outbreak found patients who developed HUS had high circulating levels of thrombomodulin and endothelin compounds released by activated or damaged endothelial cells [26].

Early diagnosis allows patients to receive early supportive therapy such as dialysis, and potentially beneficial therapy such as plasma exchange [27]. Other specific therapies are currently under development, such as an oral verotoxin-binding agent now under-

going phase III clinical trials [28]. These specific therapies offer the hope of preventing, or improving the prognosis of, HUS.

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The Central Scotland *Escherichia coli* O157:H7 Outbreak: Risk Factors for the Hemolytic Uremic Syndrome and Death among Hospitalized Patients

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Little is known about risk factors for complications of *Escherichia coli* O157:H7 infection in adults. The 1996 outbreak in central Scotland involved the largest number of adult case patients in whom hemolytic uremic syndrome (HUS) developed and, ultimately, the largest number of deaths associated with *E. coli* O157:H7 infection that has yet been recorded. We investigated risk factors for HUS in a retrospective study of all hospitalized case patients in this outbreak. Of 120 case patients, 34 had HUS develop, 28 of whom were adults. Sixteen adults died. Significant risk factors for HUS were age <15 years or >65 years (odds ratio [OR], 4.4; 95% confidence interval [CI], 1.3–14.4), hypochlorhydria (OR, 6.7; 95% CI, 1.9–24.0), and coincidental antibiotics (OR, 4.7; 95% CI 1.4–16.5). Factors associated with HUS were as follows: white blood cell count > 20×10^9 cells/L (OR, 8.25; 95% CI, 1.1–60.3), neutrophil count > 15×10^9 cells/L (OR, 8.5; 95% CI, 1.5–50.1), and serum albumin level <35 g/L (OR, 7.2; 95% CI, 1.2–42.5) ≤3 days after symptom onset. Deaths were confined to case patients >65 years of age. Early identification of risk factors for HUS is vital and could select case patients for trials of preventative and treatment therapies.

In the last decade, infection with *Escherichia coli* O157:H7 has become a significant public health problem in the developed world. In 2%–15% of cases, gastrointestinal infection progresses to the hemolytic uremic syndrome (HUS). HUS is a systemic microvascular syndrome that is initiated by secreted Shiga toxins, with predominantly renal and neurological complications, which are responsible for most deaths associated with *E. coli* O157:H7 infection. Early identification of risk factors that predict progression from gastrointestinal infection to HUS is vital because there are promising new therapies: new agents that may block Shiga toxin absorption to prevent HUS [1] and (controversially) early therapeutic plasma exchange [2].

In retrospective studies of outbreaks of *E. coli* O157:H7 infection, age (<5 years or >65 years) and elevated white blood cell count (WBC) have consistently been reported as risk factors for HUS [3–9]. There is disagreement regarding antibiotic therapy [5, 6, 10–18], therapy with antimotility agents, fever, bloody stools, vomiting, and sex as other potential risk factors in the

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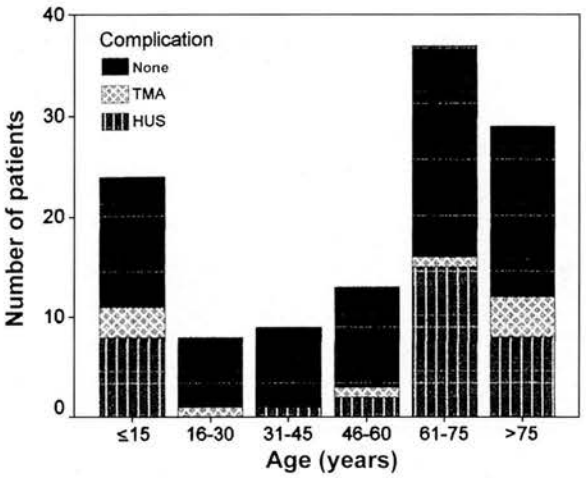

Figure 1. Age distribution of patients infected with *Escherichia coli* O157:H7 who were admitted to the hospital and who developed systemic complications. HUS, hemolytic uremic syndrome; TMA, thrombotic microangiopathy.

progression to HUS [6, 11, 12, 19, 20]. To date, most reports relate to the pediatric population, and features of adult disease are essentially unknown.

The 1996 outbreak of *E. coli* O157:H7 infection in central Scotland originated in a retail source in which cooked meats were cross-contaminated. The first group within the outbreak cohort was composed of elderly people infected at a Christmas church lunch, but ultimately the outbreak spread to affect people of all ages. The outbreak involved the largest reported number of adult case patients in whom HUS developed and, ultimately, the largest number of deaths attributable to *E. coli* O157:H7 infection that has yet been recorded from 1 outbreak. Legal proceedings delayed the collection and analysis of clinical data, but we now report a retrospective analysis of predictors of HUS and death among infected patients admitted to the hospital during this outbreak.

SUBJECTS AND METHODS

Data collection. The Information Statistics Division of the National Health Service in Scotland collated laboratory data on all confirmed, probable, and possible cases of *E. coli* O157:H7 infection identified in central Scotland during the outbreak period. For all patients who were admitted to the hospital, data were collected from case records with regard to premorbid illness, regular medications, symptoms, signs, management, and complications.

Inclusion criteria. The study included all patients with "confirmed" and "probable" cases of *E. coli* O157:H7 infection who were admitted to the hospital during the outbreak of *E. coli* O157:H7 infection in central Scotland. Patients were considered to have "confirmed" cases if the outbreak strain was isolated from a stool sample by use of standard culture with or without immunomagnetic separation [21]. Patients were considered to have "probable" cases if they had bloody diarrhea or HUS and an association with implicated food sources, but no *E. coli* O157:H7 or other organism was isolated from cultures. Lack of adequate recorded information precluded inclusion of case patients who were managed solely in the community.

Outcome measures. "Complete HUS" was defined by the following findings: evidence of red cell hemolysis (red cell fragmentation on blood film and lactate dehydrogenase level [LDH] >1.5 times the upper limit of normal), thrombocytopenia (platelet count $< 150 \times 10^9$ cells/L), and acute renal impairment (serum creatinine level >140 μM/L and rising), with or without new neurological signs (acute confusion, reduced consciousness, seizure, cerebrovascular accident). One patient, who met all other criteria, was included as having HUS despite a minimum platelet count of 228 (on death).

Analysis. Demographic characteristics, clinical symptoms and signs, antibiotic therapy, premorbid illness, regular medication, preadmission treatment, and laboratory variables were assessed in relation to outcome measures (HUS and death) by use of univariate and multivariate logistic regression analysis.

The dichotomization of continuous variables was achieved either on the basis of examination of their distribution, which yielded a natural cutoff value, or on the basis of examples of categories used in previous studies. Because of limited sample size, only variables found to be significantly associated with outcome measures in the univariate analysis were included in the multiple regression model.

Symptoms and signs were defined as those reported prior to admission or <24 h after admission to the hospital. Fever was defined as an axillary temperature >37.5°C that was recorded ≤24 h after admission. Antibiotic therapy was included if it was initiated ≤4 days after symptom onset. During the outbreak, all case patients admitted to 1 hospital were asked specifically about coincidental use of antibiotics (i.e. prescribed as treatment for another infection <4 weeks before developing symptoms related to *E. coli* O157:H7 infection) and the use of antimotility drugs. This information was not recorded at other hospitals, and analysis is confined to patients who were admitted to a hospital where the data were collected prospectively. Chronic premorbid illness and regular medication were assessed only for adults (>15 years of age), because no children had premorbid illness or were taking regular medication.

This outbreak was unique in that laboratory data were monitored for all possible case patients from presentation until at least day 14 of illness, in the hospital or in an outbreak-monitoring clinic. Levels of WBCs, neutrophils, hemoglobin, serum albumin, LDH, urea, and creatinine were assessed as predictors of HUS on the basis of results for the first blood sample ob-

Table 1. Demographic features, clinical signs and symptoms, and antibiotic therapy received for 120 patients infected with *Escherichia coli* O157:H7 who were hospitalized during the 1996 outbreak in central Scotland, with results of logistic regression analyses showing the association between these factors and hemolytic uremic syndrome/thrombotic thrombocytopenic purpura (HUS/TTP) and death.

	No. (%) of patients				No. (%) of patients aged >65 years		
Factor	Total	Who had HUS/TTP	OR (95% CI)	Adjusted OR (95% CI)	Total	Who died	OR (95% CI)[a]
n	120 (100)	34 (28)	—	—	58 (100)	16 (28)	—
Demographic data							
Age, years							
<15	24 (20)	8 (33)	3.61 (1.3–10.1)[b]	4.35 (1.3–14.4)[b]	0	0	—
15–65	38 (32)	5 (13)	1.00 (baseline)	1.00 (baseline)	0	0	—
>65	58 (48)	21 (34)	3.61 (1.3–10.1)[b]	4.35 (1.3–14.4)[b]	58 (100)	16 (28)	—
Sex							
Male	41 (34)	12 (29)	NS	—	18 (31)	7 (39)	NS
Female	79 (66)	22 (28)	NS	—	40 (69)	9 (23)	NS
Signs and symptoms							
Incubation <5 days[c]							
Yes	46 (61)	16 (35)	3.47 (1.0–11.7)	—	24 (65)	10 (42)	NS
No	30 (39)	4 (13)	1.00 (baseline)	—	13 (35)	1 (8)	NS
Bloody diarrhea							
Yes	105 (88)	31 (30)	NS	—	50 (86)	16 (32)	NS
No	15 (12)	3 (20)			8 (14)	0	
Abdominal pain							
Yes	102 (85)	30 (29)	NS	—	48 (83)	13 (27)	NS
No	18 (15)	4 (22)	NS	—	10 (17)	3 (30)	NS
Fever							
Yes	22 (18)	10 (45)	2.57 (1.0–6.7)	2.74 (1.0–7.6)	11 (19)	5 (45)	NS
No	98 (82)	24 (24)	1.00 (baseline)	1.00 (baseline)	47 (81)	11 (23)	NS
Vomiting							
Yes	59 (49)	16 (25)	NS	—	21 (36)	4 (19)	NS
No	61 (51)	18 (30)	NS	—	37 (64)	12 (32)	NS
>10 stools/day							
Yes	51 (43)	19 (37)	NS	—	24 (41)	7 (29)	NS
No	69 (47)	15 (22)	NS	—	34 (59)	9 (26)	NS
Light-headed							
Yes	7 (6)	3 (43)	NS	—	6 (10)	4 (67)	6.67 (1.1–41.0)
No	113 (94)	31 (27)	NS	—	52 (90)	12 (23)	1.00 (baseline)
Abdominal tenderness[d]							
Yes	68 (57)	19 (28)	NS	—	31 (58)	10 (32)	NS
No	51 (43)	14 (28)	NS	—	27 (42)	6 (22)	NS
Abdominal distention							
Yes	10 (8)	4 (40)	NS	—	7 (12)	4 (57)	NS
No	110 (92)	30 (27)	NS	—	51 (88)	12 (24)	NS
Tachycardia[d]							
Yes	8 (7)	6 (75)	9.33 (1.8–48.9)	7.91 (1.5–42.4)	3 (5)	1 (33)	NS
No	111 (93)	27 (24)	1.00 (baseline)	1.00 (baseline)	55 (95)	15 (27)	NS
Hypotension[e]							
Yes	4 (3)	3 (75)	NS	—	3 (5)	1 (33)	NS
No	112 (97)	30 (27)	NS	—	52 (95)	14 (27)	NS
Dehydration[d]							
Yes	16 (13)	7 (44)	NS	—	11 (19)	6 (55)	4.44 (1.1–17.6)
No	103 (87)	26 (25)	NS	—	47 (81)	10 (21)	1.00 (baseline)
Ciprofloxacin therapy	15 (13)	7 (47)	NS	—	11 (19)	5 (45)	NS

NOTE. NS, $P = .05$.

[a] Only 1 variable, dehydration, remained significant in the multiple regression, which, therefore, was not performed.

[b] Patient groups aged <15 years and >65 years were combined in logistic regression analyses for HUS/TTP.

[c] Data was missing for 44 patients; therefore, incubation period was not included in the multiple logistic regression analysis.

[d] Data was missing for 1 patient.

[e] Data was missing for 4 patients.

Table 2. Premorbid illness and regular medication of adults infected with *Escherichia coli* O157:H7 who were hospitalized during the 1996 outbreak in central Scotland, with results of logistic regression analyses showing the association between these factors and hemolytic uremic syndrome/thrombotic thrombocytopenic purpura (HUS/TTP) and death.

	Patients aged >15 years				Patients aged >65 years		
Factor	Total	Who had HUS/TTP	OR (95% CI)	Adjusted OR (95% CI)[a]	Total	Who died	OR (95% CI)[b]
n	95 (100)	25 (26)	—	—	58 (100)	16 (28)	—
Premorbid illness							
Ischemic heart disease							
Yes	32 (34)	11 (34)	NS	—	28 (48)	9 (32)	NS
No	63 (66)	14 (22)	NS	—	30 (52)	7 (23)	NS
Hypertension							
Yes	17 (18)	5 (29)	NS	—	15 (26)	1 (7)	NS
No	78 (82)	20 (26)	NS		43 (74)	15 (35)	NS
Cardiac failure							
Yes	5 (5)	1 (20)	NS	—	5 (9)	2 (40)	NS
No	90 (95)	24 (27)	NS	—	53 (91)	14 (26)	NS
Cerebrovascular disease							
Yes	15 (16)	5 (33)	NS	—	12 (21)	4 (33)	NS
No	80 (84)	20 (25)	NS	—	46 (79)	12 (26)	NS
Diabetes mellitus							
Yes	3 (3)	2 (67)	NS	—	1 (2)	0	NS
No	92 (97)	23 (25)	NS	—	57 (98)	16 (28)	NS
Hypochlorhydria							
Yes	15 (16)	9 (60)	6.00 (1.9–19.3)	6.73 (1.9–24.0)	10 (17)	6 (60)	5.70 (1.4–24.1)
No	80 (85)	16 (20)	1.00 (baseline)	1.00 (baseline)	48 (83)	10 (21)	1.00 (baseline)
Regular medication							
Aspirin							
Yes	18 (19)	6 (33)	NS	—	16 (28)	3 (19)	NS
No	77 (81)	19 (25)	NS	—	42 (72)	13 (31)	NS
ACE inhibitor							
Yes	4 (4)	1 (25)	NS	—	4 (7)	1 (25)	NS
No	91 (96)	24 (26)	NS	—	54 (93)	15 (28)	NS

NOTE. ACE, angiotensin-converting enzyme; NS, $P = .05$.

[a] Adjusted for age (i.e., the group 15–65 years of age vs. the group >65 years of age [see table 1]).

[b] Only 1 variable, hypochlorhydria, was significantly associated with outcome of death; therefore, multiple regression analysis was not performed.

tained. Because of the potentially rapid development of HUS (in 3 days, in 1 patient), only case patients from whom a first blood sample was obtained ≤48 h before the onset of symptoms were included. This restriction limited the sample size; therefore, multivariate analysis was not performed on this data. Further investigation of laboratory data (for the first 14 days of illness), in all cases, was performed by examining the mean value of variables per day after onset of symptoms in the group of patients who had HUS develop and in the group who did not. The *t* test for equality of means was applied to detect the first day in which there was a significant difference in each laboratory variable between the 2 groups.

RESULTS

In this study, 345 patients with confirmed and probable cases of *E. coli* O157:H7 infection were identified. Of those 345 patients, 279 (81%) had their cases confirmed by stool culture techniques; 120 case patients (35%) were admitted to 3 primary hospitals. It is this third group that was investigated in the regression analyses. The age range of case patients admitted to the hospital was 18 months to 94 years, with a median age of 63 years. Patients were admitted occurred from 20 November 1996 through 16 December 1996.

Complications. Thirty-four case patients had HUS: 28

Table 3. Preadmission treatment of 86 patients infected with *Escherichia coli* O157:H7 who were hospitalized during the 1996 outbreak in central Scotland, with results of logistic regression analyses showing the association between these factors and hemolytic uremic syndrome/thrombotic thrombocytopenic purpura (HUS/TTP) and death.

	No. (%) of patients interviewed				No. (%) of patients aged >65 years		
Treatment received	Total	Who had HUS/TTP	OR (95% CI)	Adjusted OR (95% CI)[a]	Total	Who died	OR (95% CI)
n	86 (100)	23 (27)	—	—	42 (100)	10 (24)	—
Antibiotic agents[b]							
Yes	14 (16)	8 (57)	5.07 (1.5–16.8)	4.71 (1.4–16.5)	10 (24)	4 (40)	NS
No	72 (84)	15 (21)	1.00 (baseline)	1.00 (baseline)	32 (76)	6 (19)	NS
Antidiarrheal agents							
Yes	26 (31)	9 (35)	NS	—	16 (38)	3 (19)	NS
No	60 (70)	14 (23)	NS	—	26 (62)	7 (27)	NS

NOTE. NS, $P = .05$.

[a] Adjusted for age (i.e., group 15–65 years of age vs. group <15 and >65 years of age [see table 1]).

[b] Received in the 4 weeks before the onset of symptoms.

adults and 6 children. HUS developed a median of 7 days (range, 3–15 days) after the onset of gastrointestinal symptoms. Twelve of 28 adults and 1 of 6 children had neurological complications of HUS, the most frequent of which was cerebrovascular accident.

Deaths. Sixteen case patients (13%) who were admitted to the hospital died; all were >65 years of age. Eleven had complete HUS, 3 had an incomplete form of HUS, and 2 had no evidence of microvascular complications. Seven of the 11 patients with HUS who died had neurological complications. The mortality rate among adults with HUS was 42% (11 of 26).

Treatment. The mainstay of treatment was supportive with correction of fluid and electrolyte imbalance. Antibiotic therapy for the treatment of gastroenteritis was prescribed according to UK guidelines [22]; therefore, ciprofloxacin was given to case patients >60 years of age who had preexisting medical conditions or who were receiving immunosuppressive therapy, acid-lowering drugs, or angiotensin-converting enzyme (ACE) inhibitors [22]. Twenty of 120 case patients received ciprofloxacin; no other antibiotic was given as primary treatment. Therapeutic plasma exchange was performed for 16 adults [2] in 1 Health Board area and 1 adult from outside that area. Five adults so treated had progressive renal impairment requiring hemodialysis. Seven children received dialysis.

Predictors of HUS

Demographic details and clinical symptoms. Childhood and old age were significantly associated with development of HUS (figure 1). HUS developed in 29 (35%) of 82 case patients <15 years or >65 years of age, compared to 5 (13%) of 38 case patients 15–65 years of age (adjusted OR, 4.4; 95% CI, 1.3–14.4; table 1). Fever (adjusted OR, 2.7; 95% CI, 1.0–7.6) and tachycardia at admission (>100 beats/min for adults and >120 beats/min for children; adjusted OR, 7.9; 95% CI, 1.5–42.4) were associated with progression to HUS. No other gastrointestinal or systemic features predicted development of HUS.

Premorbid medications and chronic illness. HUS developed in 9 (60%) of 15 adults who had low levels of gastric acid (either because they had previously undergone gastrectomy, or because they were receiving proton pump inhibitors and/or H_2-receptor antagonists; adjusted OR, 6.7; 95% CI, 1.9–24.0; table 2).

Antibiotics. HUS developed in 8 (57%) of 14 case patients who had received any antibiotic in the 4 weeks prior to the onset of symptoms related to *E. coli* O157:H7 infection (adjusted OR, 4.7; 95% CI, 1.4–16.5; table 3). HUS developed in 7 (47%) of 15 case patients who were treated with ciprofloxacin ≤4 days after symptom onset and in 26 (25%) of 104 case patients who had no antibiotic treatment, but this difference did not reach statistical significance, (OR, 2.63; CI 0.76–9.02; table 1).

Laboratory investigations. In a univariate analysis, WBC count $>20 \times 10^9$ cells/L (OR, 8.3; 95% CI, 1.1–60.3) and an absolute neutrophil count $>15 \times 10^9$ cells/L (OR, 8.5; 95% CI, 1.5–50.1) ≤48 h after symptom onset were both significantly associated with the development of HUS. Serum albumin level <35 g/L ≤48 h after symptom onset was also associated with the development of HUS (OR, 7.2; 95% CI, 1.2–42.5; table 4). Sequential analysis of laboratory results for the first 14 days of illness for all 120 patients admitted to the hospital included 901 samples (figure 2). This analysis confirmed that neutrophilia and hypoalbuminemia precede the changes in the results of laboratory tests that are regarded as indicating the development of HUS. There were significant differences in mean neutrophil count on day 2 of clinical illness between patients

Table 4. Laboratory test results obtained ≤48 h after onset of symptoms, with results of univariate logistic regression analyses showing the association between these factors and hemolytic uremic syndrome/thrombotic thrombocytopenic purpura (HUS/TTP) and death.

	No. (%) of patients			No. (%) of patients aged >65 years		
Test result	Total	Who had HUS/TTP	OR (95% CI)	Total	Who died	OR (95% CI)
n	44 (100)	9 (20)		22 (100)	6 (27)	
Neutrophil count >15 × 10^9 cells/L						
Yes	7 (16)	4 (57)	8.53 (1.5–50.1)	6 (27)	3 (50)	NS
No	37 (84)	5 (14)	1.00 (baseline)	16 (73)	3 (19)	NS
WBC count >20 × 10^9 cells/L						
Yes	5 (11)	3 (60)	8.25 (1.1–60.3)	5 (23)	3 (60)	NS
No	39 (89)	6 (15)	1.00 (baseline)	17 (77)	3 (18)	NS
Albumin level <35 g/L [a]						
Yes	9 (23)	4 (44)	7.20 (1.2–42.5)	9 (47)	4 (44)	NS
No	30 (77)	3 (10)	1.00 (baseline)	10 (53)	2 (20)	NS
Creatinine level >120 μM/L						
Yes	6 (14)	3 (50)	NS	6 (27)	2 (33)	NS
No	38 (86)	6 (16)	NS	16 (73)	4 (25)	NS
Urea concentration >10 μM/L						
Yes	8 (18)	3 (38)	NS	8 (36)	1 (13)	NS
No	36 (82)	6 (17)	NS	14 (64)	5 (36)	NS
LDH level >600 U/L [b]						
Yes	1 (3)	0	NS	1 (6)	0	NS
No	36 (97)	7 (19)	NS	16 (94)	6 (38)	NS
Platelet count <200 × 10^9 cells/L						
Yes	12 (27)	3 (25)	NS	10 (45)	2 (20)	NS
No	32 (73)	6 (19)	NS	12 (55)	4 (33)	NS
Hemoglobin level <12 g/L						
Yes	2 (5)	1 (50)	NS	2 (9)	1 (50)	NS
No	42 (95)	8 (19)	NS	20 (91)	5 (25)	NS
Sodium						
Yes	4 (9)	2 (50)	NS	4 (18)	2 (50)	NS
No	40 (90)	7 (13)	NS	18 (82)	4 (22)	NS

NOTE. LDH, lactate dehydrogenase; NS, $P = .05$.

[a] Data were missing for 5 patients.

[b] Data were missing for 7 patients.

who had HUS develop and those who did not. The traditional markers of HUS development (levels of LDH, urea, creatinine, and platelet count) became significantly different in the 2 groups 4–6 days after the onset of diarrheal symptoms. The sensitivity of a neutrophil count >15 × 10^9 cells/L as a predictor of HUS was 94%, and the specificity was 78%.

Predictors of Death

All deaths occurred in patients aged >65 years, so analysis in relation to this outcome was confined to this age group. Eleven (69%) of 16 case patients who died had complete HUS, and 3 of 16 had thrombotic microangiopathy. Dehydration and feeling light-headed on admission were the only additional prognostic variables associated with death (table 1).

DISCUSSION

E. coli O157:H7–induced HUS is associated with significant mortality, particularly among elderly patients. There is evidence that, by the time a diagnosis of HUS is made, the pathological process is well established and difficult to reverse. Because there are now promising therapies to prevent and treat HUS [1, 2], the early identification of factors that predispose to HUS or predict its development is essential.

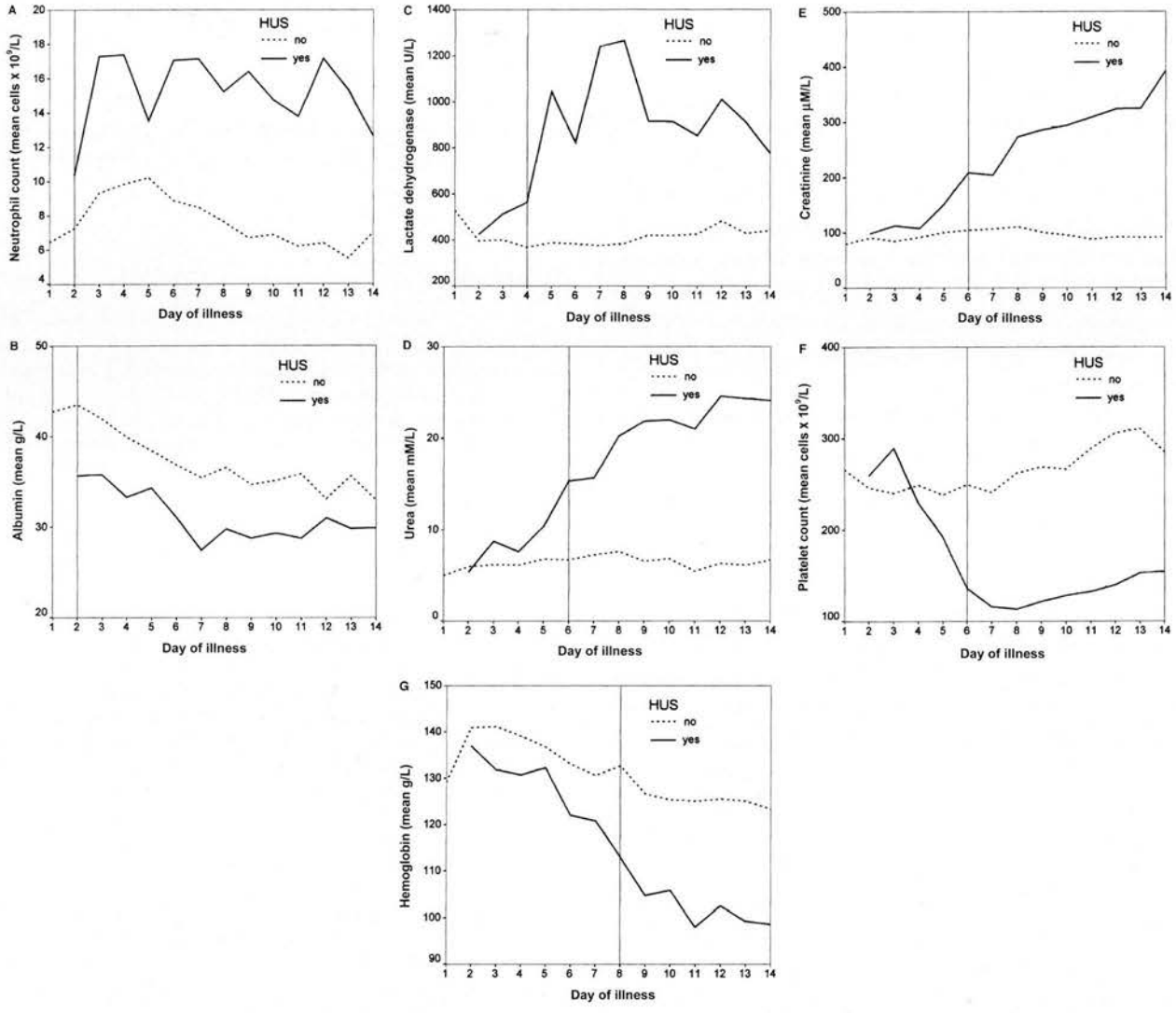

Figure 2. Mean results of laboratory tests on the day of onset of illness for patients infected with *Escherichia coli* O157:H7 who developed hemolytic uremic syndrome *(HUS)* and for those who did not.

This study included the largest number of adult patients with HUS and the largest number of deaths associated with *E. coli* O157:H7 infection to date. We found age <15 or >65 years to be the most important predictor for development of HUS. Early neutrophilia also consistently predicted the development of HUS and often preceded changes in other laboratory markers by several days. This finding reinforces speculation that neutrophils are pivotal in the pathogenesis of endothelial injury, speculation that has been validated by the recent discovery that neutrophils transport Shiga toxin [23]. Hypoalbuminemia also preceded and predicted HUS. The association of hypoalbuminemia and HUS may be a manifestation of severe gastrointestinal infection or capillary leak secondary to endothelial injury, but it may also reflect the fall in albumin seen in older people, in whom it is a well-recognized indicator of poor prognosis [24].

Observations from the earliest outbreaks and a controlled clinical trial failed to clarify the role of antibiotics in the development of HUS [5, 6, 11–13]. Retrospective analyses of the massive 1996 Sakai City outbreak in Japan contributed to this conflict: children treated early with fosfomycin had a reduced incidence of HUS [14, 15]. A prospective clinical trial involving children in the United States has demonstrated a clear association between use of sulpha-containing and β-lactam antibiotics and increased risk of HUS [18]. In vitro evidence has shown that treatment with 4-quinolones increases the release of Shiga toxin [16] and that this effect is mediated by phage replication [25]. We were, however, unable to demonstrate a significant association between early treatment with ciprofloxacin and HUS. Only a small number of selected case patients received early antibiotic therapy. We did demonstrate an association between antibiotic use that precedes the onset of

symptoms associated with *E. coli* O157:H7 infection and the development of HUS. This association has not been noted previously and may be a clinical manifestation of the effect of subtherapeutic concentrations of antibiotics. In vitro experiments have shown that subtherapeutic concentrations of antibiotics or treatment with inappropriate antibiotics increases the release of Shiga toxin [26]. It now seems likely that the effect of antibiotics on Shiga toxin production depends both on their mechanism of action and on the achievement of therapeutic concentrations. Our observations regarding treatment with ciprofloxacin and/or coincidental antibiotics advocate against the use of antibiotic therapy for *E. coli* O157:H7 infection.

The association of prior gastrectomy/acid-lowering drugs with progression from gastrointestinal infection with *E. coli* O157:H7 to HUS has not been noted previously. Patients have been shown to be at increased susceptibility to other types of bacterial gastroenteritis after gastrectomy [27], and 1 outbreak suggested that previous gastrectomy was associated with acquiring *E. coli* O157:H7 infection [5]. *E. coli* O157:H7 is an acid-tolerant organism, and hypochlorhydria was not thought to be relevant in its pathogenesis; however, there is now evidence of a wide range of acid tolerance among different isolates of *E. coli* O157:H7 [28]. An organism with reduced acid tolerance could explain our observation of more severe illness in case patients with reduced levels of gastric acid. Fever on admission was found to be associated with HUS, as it was in the 1996 outbreak in Japan [17, 20].

We have presented a detailed retrospective study of predictors of HUS and death in an outbreak of *E. coli* O157:H7 infection that had the largest number of deaths recorded to date. The methods of the study ensure that no cases were overlooked, that laboratory results and the collection of clinical data were both accurate and complete. This was, however, a retrospective study and as such may be limited by the accuracy and completeness of the medical records kept during the outbreak period.

This study has shown that mortality associated with HUS in adults remains high even with intensive management. We confirmed that age was the most important risk factor for HUS and that neutrophilia as an early predictor extended to the adult population. In addition, we identified low gastric acid and antibiotics prior to acquiring infection as risk factors. Patients can be identified who require close supervision and could potentially be entered into trials of Shiga toxin–binding agents to prevent HUS.

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Effectiveness of therapeutic plasma exchange in the 1996 Lanarkshire *Escherichia coli* O157:H7 outbreak

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Summary

Background The largest number of adult cases of haemolytic uraemic syndrome (HUS)/thrombotic thrombocytopenic purpura (TTP) during an *Escherichia coli* O157 outbreak occurred in 1996 in central Scotland. Adults who develop HUS/TTP induced by *E coli* O157 tend to be elderly and have a historical mortality rate of almost 90% when treated conservatively. Therefore the decision was made to treat adults who developed HUS/TTP during this outbreak with therapeutic plasma exchange (TPE). We report our outcome with this controversial treatment.

Methods A case definition for HUS/TTP was developed at the beginning of the outbreak. All cases meeting this definition were considered for TPE. Information on demographics, clinical features, treatment and outcome of patients was obtained by retrospective case note review.

Findings 22 adults developed HUS/TTP. They had a mean age of 71 years. 16 cases received TPE. Six cases had contraindications to TPE or died before the procedure could be done. Ten of the 22 (45%) adults with HUS/TTP died. Five of the 16 (31%) TPE-treated cases died, four of eight aged over 70 years compared with one of eight aged less than 70 years. Premorbid illness, neurological features, treatment with ciprofloxacin or prostacyclin, and the laboratory severity of HUS/TTP were not associated with death; the number of cases, however, was too small to allow statistical conclusion.

Interpretation The mortality rate is high in adults who develop HUS/TTP induced by *E coli* O157. TPE appears to be a promising treatment that was well tolerated in our elderly patients. A national register of adult cases of HUS/TTP induced by *E coli* O157 should be established.

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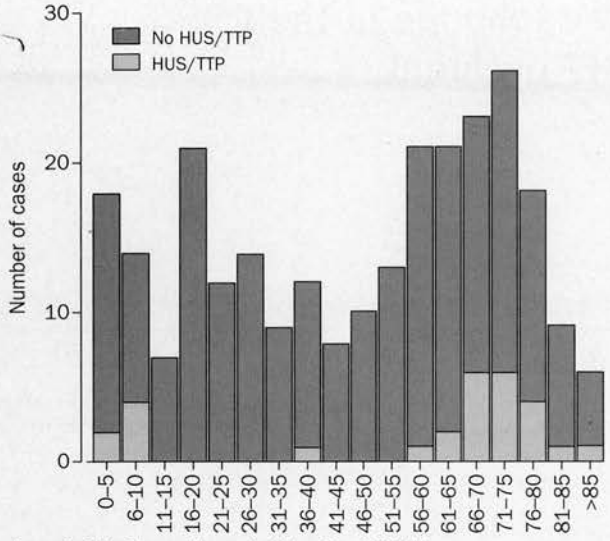
Introduction

Gastrointestinal infections with *Escherichia coli* O157 are an increasingly important public-health problem in the UK. *E coli* O157 was first recognised as a cause of gastroenteritis in 1982,[1] and the number of cases is increasing each year.[2] For the past few years the rate of infection by *E coli* O157 in Scotland has been consistently several times greater than that in the rest of the UK, and Scotland now has one of the highest rates of infection in the world.[3] In the winter of 1996 central Scotland experienced the worst outbreak, in terms of mortality, of *E coli* O157 infection ever recorded in the western world.[4]

E coli O157 infection has the potential for serious and potentially life-threatening local[5,6] and systemic complications.[7,8] The most severe systemic complications are haemolytic uraemic syndrome (HUS)—a triad of microangiopathic haemolysis, thrombocytopenia, and acute renal impairment—and thrombotic thrombocytopenic purpura (TTP)—microangiopathic haemolysis and thrombocytopenia with neurological symptoms and signs predominating over renal impairment. In the context of *E coli* O157 infection HUS and TTP are overlapping syndromes. Thrombotic microangiography—microangiopathic haemolysis and thrombocytopenia in the absence of renal or neurological disorders—is the earliest feature of the range of disease that leads to HUS/TTP. All are a consequence of the same pathological microvascular process mediated by the *E coli* O157 shiga toxins.[9] Risk factors associated with the development of systemic complications have been identified, the most consistent of which are extremes of age (<4 years[10,11] and >65 years[12,13]) and raised white-blood-cell count.[14]

A general consensus still supports the use of therapeutic plasma exchange (TPE) in adults with idiopathic HUS and in patients of all ages with TTP.[15,16] This treatment is controversial for *E coli* O157 infection, since experience of TPE in the treatment of HUS/TTP induced by this organism is limited. The outbreak in central Scotland of *E coli* O157 infection centred on the Lanarkshire town of Wishaw where the implicated retail outlets were located. The largest cohort within the outbreak was a group of elderly people who attended a Christmas church lunch. The elderly are known to be at particular risk of developing HUS/TTP and the expected mortality rate in those treated conservatively is 88%.[13] We therefore decided early in the course of the Lanarkshire outbreak to use TPE in the treatment of adults who developed HUS/TTP.

Legal proceedings surrounding the 1996 outbreak have delayed the compilation of clinical reports. We now report the clinical outcomes with TPE in adults who



Age distribution of Lanarkshire *E coli* O157 cases

developed HUS/TTP in Lanarkshire during this outbreak.

Patients and methods

The Information and Statistics Department of the Scottish Home and Health Department collected data on the demographics and laboratory results of all possible outbreak cases. We collected clinical data by reviewing the case notes of all cases admitted to hospital in the Lanarkshire area.

All confirmed or probable cases of *E coli* O157 infection, identified in the Lanarkshire area during the outbreak period, were included in the assessment and analysis. Confirmed cases were those in whom the outbreak strain of *E coli* O157 was isolated from stool samples. If stool cultures were negative at the local laboratories, specimens were sent to Scotland's *E coli* reference laboratory in Aberdeen, for the more sensitive isolation method of immunomagnetic separation.[17] Probable cases were those with bloody diarrhoea or HUS/TTP, an association with food sources implicated in the outbreak, no *E coli* O157 isolated, and no other organism isolated. Adults were defined as patients 15 years of age or older.

To allow standardisation of diagnosis in the face of a huge clinical workload, a case definition for HUS and TTP was developed at the beginning of the outbreak. HUS was defined as evidence of red-cell haemolysis (red-cell fragmentation on blood film and lactate dehydrogenase >1·5 times the upper limit of normal [our laboratory 0-480 IU/L]) plus thrombocytopenia (platelets $<150\times10^9$/L) with rising urea and creatinine concentrations. All three criteria had to be met before the diagnosis could be made, but not necessarily on the same blood sample. A diagnosis of TTP was given to patients who met these laboratory criteria and developed new neurological symptoms and signs. One patient was included as having developed HUS despite a minimum platelet count of 228×10^9/L (on death). He had bloody diarrhoea, an association with an implicated food source, acute renal failure, the criteria for red-cell haemolysis, and a falling platelet count.

In the assessment of premorbid illness, medical histories included as relevant were ischaemic heart disease, cardiac failure, hypertension, cerebrovascular disease, renal disease, diabetes, and immunosuppression. Pulmonary oedema was diagnosed on clinical and radiological evidence.

TPE was performed at three centres with three Cobe Spectra Apheresis Systems (Cobe Laboratories Ltd, Gloucester, UK) and a Baxter Fenwal CS-3000 Plus Cell Separator (Baxter Healthcare, Newberry, UK). Plasma was exchanged with 2·0–2·4 L fresh frozen plasma or cryosupernatant in refractory patients.[18] The anticoagulant used was ACD-A. A combination of central and peripheral venous access was used. Intravenous hydrocortisone was given with each exchange. Intravenous prostacyclin was also given to cases receiving TPE, at doses between 40 mg/h and 200 mg/h, where tolerated. Data were analysed by means of SPSS (version 7.5).

Results

There were 262 cases of *E coli* O157 infection in the Lanarkshire area: 200 confirmed cases and 62 probable cases. The median age of all affected was 53 years, but there were higher numbers at the extremes of age (figure). 47% (124/262) of infected individuals were over 55 years of age. 13 (5%) people died. In 10 cases death was associated with the systemic complications of *E coli* O157 infection.

28 (11%) of the Lanarkshire cases of *E coli* O157 met the diagnostic criteria for HUS/TTP. Cases met the criteria for HUS/TTP a median of 7 days (range 4–15) after the onset of gastrointestinal symptoms. A further eight cases had evidence of thrombotic microangiopathy but did not meet the criteria for HUS/TTP and were not eligible for TPE. 22 (79%) cases with HUS/TTP were

Patient	Age (years)	Sex	TPE	Prosta-cyclin	Ciprofloxacin	Dialysis	Neurological features	Medical history	Pulmonary oedema	Urea (mmol/L)	Creatinine (μmol/L)	Neutrophil count ($\times10^9$/L)	Platelet count ($\times10^9$/L)	LDH (U/L)	Haemoglobin (g/dL)	Death
1	40	F	Yes	Yes	Yes	Yes	..	Yes	Yes	11·9	242	31·1	150	1049	8·9	..
2	60	F	Yes	Yes	..	Yes	Yes	..	Yes	11·2	215	24·6	64	1151	9·2	..
3	62	F	Yes	Yes	..	..	..	Yes	Yes	18·8	141	3·7	32	1028	10·5	..
4	63	F	Yes	Yes	..	..	..	Yes	..	14·6	115	10·3	170	1065·	9·4	..
5	66	F	Yes	..	..	Yes	..	..	..	34·9	272	14·3	58	1812	10·3	..
6	69	M	..	..	Yes	..	..	Yes	Yes	16·2	187	28·7	250	1043	11·2	Yes
7	69	M	Yes	Yes	..	..	Yes	Yes	Yes	12·2	150	14·7	95	1052	16·0	..
8	70	M	..	..	..	..	Yes	..	Yes	18·9	105	9·2	145	555	12·7	Yes
9	70	F	Yes	..	..	..	..	..	Yes	13·2	162	23·0	83	939	10·0	..
10	70	M	Yes	Yes	Yes	Yes	..	..	Yes	20·7	259	12·2	166	765	13·9	Yes
11	71	F	Yes	Yes	..	..	..	Yes	..	13·7	114	6·9	166	1153	12·8	..
12	71	F	Yes	..	Yes	..	Yes	Yes	Yes	8·6	139	22·4	85	1268	13·3	..
13	71	F	..	..	Yes	..	Yes	..	..	34·3	268	32·9	97	1410	11·2	Yes
14	72	F	Yes	..	Yes	..	Yes	..	Yes	20·2	177	21·8	29	1971	11·9	Yes
15	74	M	Yes	Yes	..	..	Yes	Yes	Yes	23·4	196	8·7	115	1150	8·0	..
16	74	M	Yes	..	..	..	..	Yes	Yes	15·0	219	4·9	86	1349	12·0	Yes
17	76	M	..	..	..	..	Yes	..	..	33·7	116	14·2	148	676·	12·7	Yes
18	78	F	Yes	..	..	..	Yes	Yes	Yes	36·4	313	29·6	237	1112	12·1	Yes
19	79	F	..	..	Yes	..	Yes	Yes	..	17·9	158	12·9	121	799	13·5	Yes
20	80	F	Yes	Yes	..	..	..	..	..	12·2	123	9·2	125	841	10·4	..
21	83	F	Yes	..	Yes	..	Yes	Yes	..	11·5	193	14·9	129	1729	11·2	Yes
22	90	F	..	..	Yes	..	Yes	Yes	Yes	32·6	269	12·5	121	903	14·1	..

Blood results taken from the day that criteria for HUS/TTP were reached, before TPE in cases so treated.

Table 1: **Adult HUS/TTP cases**

Patient	Reason
6	Cardiac arrest before transfer for plasma exchange
8	Diagnosis not suspected
13	Cardiac arrest before transfer for plasma exchange
17	Fluid overload, pneumonia, septicaemia
19	Acute abdomen, dementia, relatives' wish
22	Fluid overload, precarious fluid balance

Table 2: **Reasons for patients not receiving TPE**

adults and six (21%) were children. The median age of adults who developed HUS/TTP was 71 years and the median age of children 6 years. The demographics, clinical features, treatment, laboratory results, and outcome of the adult cases with HUS/TTP are shown in table 1. Blood results are taken from the day that the diagnostic criteria for HUS/TTP were met, before TPE in cases so treated.

The mortality rate in adults with HUS/TTP was 45% (ten of 22). Seven of 12 cases aged over 70 years and three of ten aged 70 years or less died. There were no deaths in children. Necropsies were done for all cases who died. Causes of death in patients with HUS/TTP were acute renal failure secondary to HUS (two cases), cardiac arrest (two cases), intracerebral haemorrhage, cerebral infarction, acute myocardial infarction, multiple organ failure, hepatorenal syndrome secondary to macronodular cirrhosis and septic shock.

TPE was used in 16 of the 22 adult patients with HUS/TTP. For patients treated with TPE later received haemodialysis, because of deteriorating renal function. Patients who did not receive TPE were either too unwell to tolerate the procedure or died before TPE could be carried out (table 2).

In all 16 cases treated with TPE, the first exchange was first done within 24 h of the criteria for HUS/TTP being met. The minimum number of changes was one, the maximum 16, and the median six. Patients underwent a total of 107 procedures, and 1100 units of fresh frozen plasma were used. Two patients proved refractory to treatment with fresh frozen plasma, after five and six exchanges, but were successfully treated by additional TPE with cryosupernatant as the exchange fluid.

Five of the 16 (31%) TPE-treated patients died, four of eight aged over 70 years and one of eight aged 70 years or less. Premorbid illness, neurological features, treatment with ciprofloxacin or prostacyclin, and the laboratory severity of HUS/TTP were not associated with death, although the number of cases was too small to allow statistical conclusion.

The most frequent complication associated with plasma exchange was pulmonary oedema, which was diagnosed on clinical and radiological grounds in 11 cases. Pulmonary oedema was not confined to patients undergoing TPE; three of six HUS/TTP cases not treated with TPE had pulmonary oedema. Hypocalcaemia (calcium <2·12 mmol/L) occurred in 15 of the 16 patients treated with TPE. Although severe (minimum serum calcium 1·32 mmol/L) in many cases, intravenous calcium supplements were given when appropriate and no clinical manifestations of hypocalcaemia were observed. Hypomagnesaemia (magnesium <0·7 mmol/L) occurred in eight patients; intravenous magnesium was given as appropriate and no clinical effects were observed. Other complications associated with TPE were line infection with meticillin-resistant *Staphylococcus aureus* and extravasation infusion.

Discussion

HUS/TTP used to be a rare disease in adults, with an estimated frequency of one case per million per year.[19] In 50% of cases it was associated with pregnancy, malignant hypertension, HIV infection, cancer, or chemotherapy, and the remainder of cases were familial or of unknown cause. In 1986 the first association of HUS/TPP with *E coli* O157 infection was made[7] and the incidence of the disorder has since continued to rise in parallel with the global rise in *E coli* O157 infections.[2] After exposure to *E coli* O157, between 3% and 7% of all patients progress to overt HUS/TTP.[20] The incidence of HUS/TTP is highest in children and elderly people.[20]

The course and prognosis of HUS/TTP differ substantially between adults and children. Children with HUS develop acute renal failure precipitately and the treatment of choice is dialysis, which is initiated when the child becomes oliguric. Most children respond to dialysis, and mortality rates of less than 5% are now reported.[21] In the central Scotland outbreak there were no deaths in children. Adults seem to develop neurological or cardiovascular complications before the onset of oliguria. Neurological features are associated with increased mortality,[22] and neurological and cardiovascular complications of HUS/TTP were the most frequent causes of death in the central Scotland outbreak.

The only other reported experience of HUS/TTP induced by *E coli* O157 in adults of which we are aware was from a Canadian nursing-home outbreak in 1985.[13] In that outbreak 11 of 12 patients who developed HUS/TTP died, compared with ten of 22 in the central Scotland outbreak. The patients who developed HUS/TTP in the nursing-home outbreak were slightly older (mean age 83 years) and probably frailer than our patients. All patients from the nursing-home outbreak were admitted to hospital and treated with full supportive therapy, but without TPE. We believe that our use of TPE was the only difference in treatment between our patients and those reported from the Canadian nursing-home outbreak. Also, in our patients, we can find no evidence of any other treatment influencing outcome from HUS/TTP. We therefore suggest that without the use of TPE the proportion of HUS/TTP cases who died would have been higher.

When HUS/TTP develops secondary to gastrointestinal infection with *E coli* O157, endothelial damage is mediated by the lysogenic-phage-encoded shiga toxins 1 and 2.[23] The shiga toxins enter the systemic circulation causing microvascular damage at target organs by a process that is thought to be mediated by proinflammatory cytokines such as tumour necrosis factor α and interleukins.[24] Endothelial damage induces the formation of large von Willebrand factor multimers,[25] which in turn may cause platelet aggregation with the formation of small-vessel thrombi in target organs. These combined processes are manifest clinically as thrombocytopenia, haemolytic anaemia, renal impairment, and neurological features such as confusion, seizures, and cerebrovascular accidents. The theoretical basis for use of TPE in *E coli* O157 infection is that it may remove the shiga toxins, proinflammatory cytokines, and von Willebrand factor multimers from the circulation, thereby removing the factors that initiate and perpetuate the microvascular process that leads to HUS/TTP.

Plasma exchange is an expensive (£2500 per person treated in our hospital) and intensive procedure. Its effectiveness in the treatment of HUS/TTP induced by *E coli* O157 needs to be shown definitively in a multicentre, randomised controlled trial. However, for a disease with very high mortality and just one potentially beneficial treatment option, a trial that withholds this option would be hard to justify. It would also be extremely difficult to organise since cases of *E coli* O157 occur sporadically. There will always be an unavoidable selection bias within such a trial, with patients who are excluded from treatment because they have contraindications to TPE or who die before treatment can be initiated.

If 5% of all cases of *E coli* O157 develop HUS/TTP, we would expect about 40 adult cases of HUS/TTP per year in the UK (data from the Communicable Disease Surveillance Centre and Scottish Centre for Infection and Environmental Health). We suggest that a national register be established for adult cases of HUS/TTP, as currently operates for cases in children. This database would enable monitoring of treatment and outcomes in adults, providing definitive evidence of the effectiveness of TPE within about 5 years.

There is no evidence from our experience that TPE is harmful. A national register of HUS/TTP secondary to *E coli* O157 could define the role of TPE in the treatment of this serious disorder.

Contributors

S Dundas was responsible for data collection, data analysis, and writing of the final paper. J Murphy and G Jones were responsible for provisional data collection and initial presentation of the data. J Murphy, W T A Todd, R L Soutar, and G Jones were responsible for managing the cases during the outbreak, standardising the inclusion criteria for therapy, recording data, the design of the paper, and revision at all stages. S J Hutchinson gave statistical advice.

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CONCISE COMMUNICATION

Blood Group and Susceptibility to Disease Caused by *Escherichia coli* O157

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Patients ($n = 186$) infected during the *Escherichia coli* O157 outbreak in Scotland in 1996 were assessed for blood group markers (ABO, Lewis, and P) associated with other gastrointestinal infections. Binding of bacteria to epithelial cells was assessed by flow cytometry. Buffy coats from blood donors were examined for inflammatory responses to culture filtrates of the outbreak strain. Individuals of blood group O comprised 63.4% of patients, compared with 53.4% ($P < .05$) and 53.9% ($P < .01$) of neighboring populations in Airdrie and Glasgow, respectively; group O also comprised 64.3% of patients with hemolytic uremic syndrome (HUS) and 87.5% of patients who died ($P < .05$). No or weak agglutination by anti-P antiserum was observed for 40.7% of control persons ($n = 122$), 61.5% of all patients ($P = .0027$), and 83.3% of patients with HUS ($P = .013$). The susceptibility of group O to *E. coli* was not associated with increased binding of bacteria to epithelial cells or with higher production of tumor necrosis factor (TNF)–α or interleukin-6. Leukocytes of P-negative blood donors produced higher levels of TNF-α than those of P-positive donors.

Morbidity and mortality associated with the outbreak of *Escherichia coli* O157 infection in central Scotland in 1996 raise the question of why some individuals exposed to this organism developed severe disease and others did not. Aside from the extremes of age in those infected, few demographic factors have been associated with susceptibility to this disease. In relation to severity of disease, we have identified neutrophilia or hypoalbuminemia in an individual at hospital admission as important risk factors for hemolytic uremic syndrome (HUS) [1]. Identification of risk factors has clinical relevance, such as definition of patients who require intensive monitoring and supportive treatment, and, controversially, definition of individuals in whom the use of therapeutic plasma exchange (TPE) may prevent progression to HUS [2].

Studies of other enteric pathogens indicate that blood group is a risk factor for some of these diseases. Occurrence or severity of diarrheal disease due to *E. coli* [3], *Vibrio cholerae,* and *Helicobacter pylori* is associated with 2 genetic characteristics: the O blood group and the inability to secrete the water-soluble glycoprotein forms of one's ABO blood group antigens (nonsecretion) [4–6]. The P blood group, characterized by a receptor on endothelial surfaces for the Shiga toxin (ST) [7], may be a third genetic factor, but there have been conflicting reports on association between P blood group and susceptibility to disease due to *E. coli* O157 in white populations [8, 9].

Our earlier work [4] demonstrated that *H. pylori* bound in higher numbers to cells of individuals of blood group O and that binding of the bacteria was significantly associated with expression of H type 2, the antigen of blood group O. In addition, individuals of blood group O produced significantly higher levels of tumor necrosis factor (TNF)–α and interleukin (IL)–6 in response to *H. pylori* [10].

The aims of the study were as follows: (1) to assess ABO and P blood groups and secretor status among patients with disease due to *E. coli* O157, in comparison with the population in central Scotland; (2) to determine whether the H type 2 antigen is a receptor for the outbreak strain or other strains of *E. coli* O157; and (3) to assess inflammatory responses to a culture filtrate of the outbreak strain in relation to ABO and P blood groups.

Subjects and Methods

Subjects. There were 186 patients in the study who developed disease due to confirmed *E. coli* O157 infection during the 1996 outbreak in Lanarkshire. For each patient, 10 possible age- and sex-matched control subjects were identified by computer selection

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Ethical approval of the study was obtained from the Lanarkshire Health Board research ethics committee. Informed consent was obtained from each subject, and human experimentation guidelines of the institutions involved were followed.

There is no commercial interest or association that might pose a conflict of interest.

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from data generated from the Lanarkshire Community Health Index (CHI), and, with their general practitioner's consent, they were invited to participate. Of these, 122 agreed to participate and are included as control subjects. Blood was drawn from patients at the time of infection and was drawn from patients and control persons 1 year after infection. Information on ABO but not Lewis or P blood groups was available for 16 individuals who died in Lanarkshire hospitals, 12 in Monklands District General Hospital in Airdrie and 4 in Law Hospital in Carluke. Results for patients and CHI control persons were also compared with data obtained for earlier studies in central Scotland [11, 12].

Patients with clear evidence of red blood cell (RBC) hemolysis were considered to have ST-mediated thrombotic microangiopathy (TMA), a proportion of whom progressed to HUS. HUS was defined by the following findings: RBC hemolysis (RBC fragmentation on blood film and lactate dehydrogenase level >1.5 times the upper limit of normal); thrombocytopenia (platelet count $<150 \times 10^9$/L); and acute renal impairment (serum creatinine level >140 μmol/L and rising).

Determination of blood groups. ABO blood group was determined by slide agglutination with monoclonal anti-A and anti-B reagents from the Scottish National Blood Transfusion Service (SNBTS). Lewis antigens were determined by tube agglutination with monoclonal anti-Lewis^a and anti-Lewis^b reagents (SNBTS). Polyclonal anti-P typing serum (SNBTS) was used in slide agglutinations, with results recorded as follows: 3, strong; 2, moderate; 1, weak; and 0, no visible agglutination. The cells also were incubated with the anti-P serum for 30 min at room temperature, with agglutination assessed by flow cytometry (Coulter) in comparison with cells incubated for 30 min with PBS. Secretor status was assessed by hemagglutination inhibition tests, and results were compared with results for Lewis^a and Lewis^b antigens by ELISA for Lewis antigens in saliva.

Bacteria. In addition to the outbreak strain, 8 additional strains of *E. coli* O157 of different phage types were obtained from Dr. M. F. Hanson (Central Microbiological Laboratory, Western General Hospital, Edinburgh). The bacteria were cultured in category 3 high-containment facilities, and formalin-fixed cultures were used in the binding assays.

Binding of bacteria to epithelial cells. Binding of bacteria to buccal epithelial cells of the different ABO blood groups and of the Kato III gastric epithelial cell line was assessed by the flow cytometry method described elsewhere [4]. Blood group antigens were detected with monoclonal antibodies to H type 2, A, B, Lewis^a, and Lewis^b by flow cytometry. Bacterial binding was examined in relation to the level of expression of blood group antigens, as reflected by the level of monoclonal antibodies bound [4].

Screening for bacterial adhesins that bind blood group antigens. The spectrophotometric method for detection of direct binding of biotinylated synthetic blood group oligosaccharides (Syntesome) was used [4]. Binding of fluorescein-labeled blood group oligosaccharides to the bacteria was also examined by flow cytometry. *H. pylori* strain NCTC 11637 [4], which expresses fucose-binding adhesins, was included in each experiment as a positive control.

Inflammatory responses to E. coli *O157 antigens.* Inflammatory responses elicited from leukocytes by a sterile culture filtrate of *E. coli* O157 were assessed by the blood group and P antigen level of the donor. The bacteria were grown in nutrient broth for 24 h at 37°C in universal containers. The bacteria and filtrates were prepared in a category 3 high-containment facility. Buffy coats from blood donors ($n = 32$) were obtained from the Blood Transfusion Service, Royal Infirmary of Edinburgh. There were 8 donors each of A, B, O, and AB blood groups, and the levels of IL-6 and TNF-α were assessed by ELISA and bioassay, respectively [13].

Statistical analyses. Samples were coded so that the blood groups were determined without reference to patient or control group. Results were entered into a database and were assessed by use of a statistical software package (Epi Info). Prevalence was compared between groups by χ^2 tests with Yates's correction, and confidence intervals (CIs) for odds ratios were calculated by exact methods.

Results

Epidemiological study. ABO and P blood groups were determined for 186 patients infected during the Lanarkshire outbreak and for 122 control persons obtained through the CHI. Because the proportion of blood group O among the CHI control persons was higher than that among the population covered by the Lanarkshire Blood Transfusion Service based at Law Hospital (51%; K. Liddle, personal communication), data for ABO blood groups also were compared with data from our earlier study on an outbreak of meningococcal disease at the Airdrie Academy [11] and data for blood donors in greater Glasgow [12]. There was no significant difference between the proportion of blood group O among patients (63.4%) and that among the CHI group (58.3%); however, compared with the larger groups, Airdrie ($n = 371$; 53.4%; $P < .05$) and Glasgow ($n = 5898$; 53.9%; $P < .01$), there was a significantly increased proportion of blood group O among the patients. The proportion of patients with HUS who were blood group O was 18 (64.3%) of 28; however, among patients who died, 14 (87.5%) of 16 were blood group O, and this was significantly increased, compared with all 3 control groups ($P < .05$; figure 1). No differences in the proportions of secretors and nonsecretors of ABO blood group antigens were observed between patient and control groups.

RBC samples from 83 (61.5%) of 135 patients who attended follow-up clinics were not agglutinated or were only weakly agglutinated by the anti-P antiserum, compared with 46 (40.7%) of 113 samples from the CHI control persons ($P = .0017$; 95% CI, 1.35–4.00). Among 17 patients who attended the follow-up clinics and who developed TMA, 14 (82.4%) had no or only weak reactions to anti-P antiserum, compared with 46 (40.7%) of the 113 CHI control persons ($P = .0032$; 95% CI, 1.73–38.41). Among patients with TMA, 12 developed HUS, and 10 (83.3%) had no or only weak reactions to anti-P, compared with 46 (40.7%) of the 113 CHI control persons ($P = .0130$; 95% CI, 1.43–70.37). Data on P blood group were not available for patients who died.

Bacterial binding in relation to blood group and secretor status. Nine strains of *E. coli* O157, including the outbreak strain, were

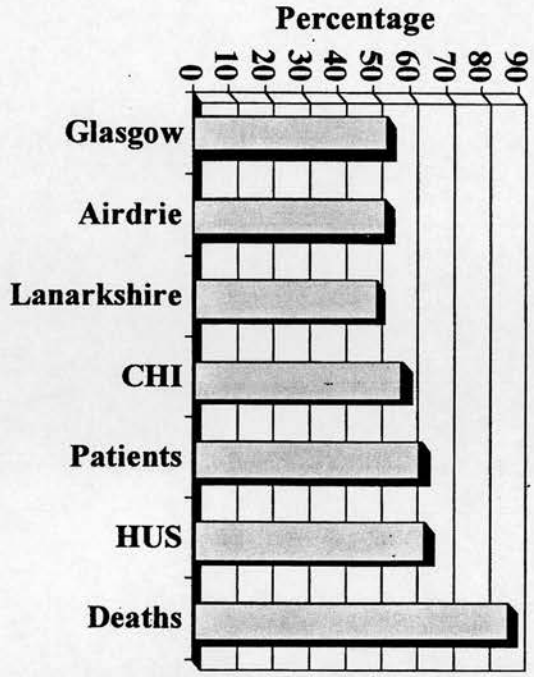

Figure 1. Percentages of blood group O individuals in control and patient groups. CHI, Community Health Index (Lanarkshire); HUS, hemolytic uremic syndrome.

tested for binding to epithelial cells of secretor and nonsecretor donors of blood groups A, B, O, and AB. There was no correlation between binding of the bacteria to the Kato III cell line or epithelial cells from the donors and expression of ABO or Lewis blood group antigens.

Binding of labeled blood group oligosaccharides to E. coli *O157.* None of the strains bound any of the biotinylated or fluorescein-labeled ABO or Lewis blood group antigens. However, the oligosaccharides bound to the *H. pylori* control in the assays.

Inflammatory responses and blood group. No significant differences in levels of either TNF-α or IL-6 were associated with ABO blood group. With respect to P antigen effect on the RBCs, there were no differences in IL-6 levels. There were 8 donors whose RBCs were not agglutinated by anti-P, and 5 of these blood samples produced TNF-α levels >100 IU/mL in response to the culture filtrate. None of the donor RBC samples expressing P antigen produced TNF-α responses approaching 100 IU/mL ($P = .001$).

Discussion

Among patients infected by *E. coli* O157 during the Lanarkshire outbreak, the proportion who were blood group O was 63.4%, which was significantly higher than that of the general population in the surrounding area (51%–53.3%) but not significantly higher than that of the internal control group of 122 individuals selected from the CHI. The proportion of blood group O individuals who died during the outbreak was significantly increased, compared with all 3 control groups. In contrast to our earlier studies on *H. pylori* [4, 10], the increased susceptibility of blood group O individuals to disease due to *E. coli* O157 was not associated with increased binding of bacteria to epithelial cells of blood group O or to higher inflammatory responses of blood group O leukocytes.

The high proportion of patients with disease due to *E. coli* O157 (61.5%) whose erythrocytes were not agglutinated or only weakly agglutinated by anti-P antiserum, particularly those who developed TMA (82.4%) or HUS (83.3%), supports the findings obtained for 32 children with HUS, among whom there was a significant excess of patients with weak or absent expression of P1, particularly among those with poor disease outcome [8]. Unfortunately, there is no information on the P blood group for patients who died. Two possibilities are suggested. The P antigen on erythrocytes can absorb the ST and prevent it from reaching target cells in the kidney or brain, or ST and/or endotoxin in the culture filtrate is better able to bind to leukocytes of individuals who do not express P antigen or have low levels of this antigen on their cells.

TNF-α has been implicated as a major factor in tissue damage in an animal model of *E. coli* O157 [14]. High levels of TNF-α, IL-6, IL-8, IL-10, and IL-1 receptor antagonist have been reported among patients infected with *E. coli* O157 [15]. The induction of very high levels of TNF-α by the culture filtrate from leukocytes of P-negative individuals is an observation to be pursued. The results of these studies complement findings of an increased proportion of individuals with low levels of P antigen on their RBCs in the patient group, particularly among the patients who developed HUS. This observation might help identify individuals at greater risk of more-serious disease caused by *E. coli* O157 and, in particular, those who would benefit from TPE [2].

This study identified 2 genetic risk factors for disease due to *E. coli* O157. Although the original hypothesis that individuals of blood group O might be at increased risk of disease was supported by the data, the variations in levels of the P blood group antigen appear to be more important in relation to severity of disease. These findings provide new avenues for investigation of the differences in host susceptibility factors and responses that contribute to development of serious kidney and vascular damage in response to these infections.

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